School of Land and Food

The influence of fertiliser type on mycorrhizal colonisation and plant growth in horticultural systems

By

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Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution and to the best of my knowledge contains no material previously published or written by any other person, except where duly acknowledged in the thesis.

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Abstract

Conventional synthetic fertilisers are widely used in many horticultural systems and while there is an increasing interest in alternative nutritional management strategies which better promote soil health, there is a gap in studies which objectively compare many of these alternatives. Previous research has highlighted the benefits of alternative nutrient management strategies on soil biota, including mycorrhizal fungi which are important symbionts of many crops. To investigate the difference between conventional fertilisers and a variety of alternative nutrient sources on mycorrhiza and plant growth, research trials were conducted in two systems, sunflower and perennial deciduous tree crops, over three growing seasons.

The effect of nutrient source and bio-inoculants on productivity of sunflower was examined in pot trials under glasshouse conditions in three separate experiments. Firstly, inoculation with "effective microbes" (EMs) was examined with an organic humate based soil amendment with and without inorganic fertiliser. Secondly, inoculation with a commercial spore preparation of arbuscular mycorrhizal fungi (AMF, MYCORMAX™) was used in conjunction with a range of fertiliser treatments including a liquid organic fertiliser (LOF) and a liquid inorganic fertiliser (LIF) to test sunflower productivity, mycorrhizal colonisation and nutrient uptake. Thirdly, a range of organic soil amendment treatments (Ferbon®, compost, soluble humate granules (SHG)) were examined in conjunction with the same AMF spore inoculation to further explore the factors described above. In the deciduous tree crops of apples and cherries, the influence of alternative orchard floor management strategies on AMF colonisation, and the availability of nutrients in the soil and leaf nutrient status were examined. Additionally, the response of AMF colonisation to conventional and alternative nutrient regimes, and its correlation with the flavour development of apple and cherry fruit were investigated.

Inoculation with EM significantly increased sunflower plant height, number of nodes, stem diameter and early flowering compared to non-inoculated plants but significantly reduced leaf chlorophyll content when nutrient deficiency was observed. Soil amendment with lignite-based humates such as Ferbon® led to an increase in plant height and flower diameter compared to control plants, but did not influence leaf chlorophyll content or number of nodes. The findings demonstrated that, with the availability of sufficient accessible nutrients, both EM and lignite-based humates have the ability to increase plant productivity.

AMF inoculation in combination with LIF increased sunflower plant height, stem diameter and leaf chlorophyll content. Sunflower growth attributes were only slightly influenced by AMF with LOF. Node number and flower head diameter also significantly increased with AMF colonisation. Use of LOF as a nutrient source increased concentration of foliar P, K and B and percentage of AMF colonisation.

Sunflower productivity was affected positively by AMF inoculation and soil amendment with organic supplements. AMF inoculation with compost greatly increased plant height while AMF and SHG increased both leaf chlorophyll content and stem diameter. Positive relationships were found between AMF inoculation plus organic supplements and nutrient uptake by plants as well as nutrient release in the soil. The inoculation of AMF plus SHG significantly increased leaf nutrient levels of Ca, Mn and Zn in plants grown in an orchard sourced soil compared to those grown in forest sourced soil.

In the perennial tree crop studies, nutrient regime had no impact on colonisation of tree roots by resident AMF species. Biochar applied as a soil amendment led to significantly increased AMF colonisation, while planting a combination of clover/grass in the tree row increased the presence of AMF arbuscules and vesicles. In the two cherry sites, mycorrhizal colonisation was not affected by the nutrient regime. An interaction between nutrient regime and EM was observed, with EM application increasing colonisation in 'Lapin' cultivar roots when applied to alternative regime plots, but reducing colonisation in conventional plots. However the reverse occurred in the 'Sweetheart' cultivar roots. This difference between the two cultivars is most likely influenced by the different soil types in these two orchards. Properties of both apple and cherry fruit were influenced by AMF colonisation with a positive correlation between colonisation and both total soluble solids (TSS) and titratable acidity (TA) but a negative correlation with the ratio of TSS/TA. Soil nutrient levels were adequate in the alternative plots where humates were applied, and levels of some nutrients were higher in these plots than in the conventional plots. Hence it can be concluded that nutrient regimes based on application of humates, particularly in conjunction with biochar or EM inoculation have the ability to improve soil nutrient uptake, leaf nutrient levels and fruit properties in the presence of mycorrhizal activity.

The studies reported in this thesis demonstrate that inoculation with EM and/or AMF can have significant effects on plant growth, and that, in the presence of an appropriate level of nutrients, EM and humates have the ability to increase sunflower productivity. These studies have also demonstrated that orchard floor management has a greater impact on AMF

colonisation than fertiliser regime. The degree of AMF colonisation and its interaction with both fertiliser applications and orchard floor management are strongly correlated with fruit quality, but not always associated with plant growth.

Co-authorship statement

This thesis comprises of work, which has been published or prepared to be submitted to journals. Information for each chapter is provided in the section of communications arising from this thesis.

The following people contributed to the publication and preparation of the work undertaken as part of this thesis:

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Chapter 4: Effect of fertiliser type and mycorrhizal inoculation on growth and development of sunflower.

Chapter 5: Effects of organic supplements, AMF inoculation and soil type on sunflower growth.

Chapter 6: Changes in arbuscular mycorrhizal colonisation with different nutrient and orchard floor management practices and relationship to flavour of apple and cherry fruit.

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We the undersigned, do hereby agree with the above stated "proportion of work undertaken" for each of the above published, peer-reviewed manuscripts (or ready-to-be-sent papers) contributing to this thesis:

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List of Abbreviations

ALT	alternative
AMF	arbuscular mycorrhizal fungi
AT	after transplanting
BC	biochar
CC	cover crops (clover/grass)
COM	compost
CON	control
CONV	conventional
EM	effective microbes
FER	Ferbon
HERB	herbicide
LIF	liquid inorganic fertiliser
LOF	liquid organic fertiliser
LSD	least significant difference
MA	malic acid
ML	megalitre
nd	not detected
ns	not significant
P	probability
SHG	soluble humate granules
SA	see appendices
TA	titratable acidity
TSS/TA	total soluble solids/ titratable acidity ratio

"Chapter 1" General Introduction

The studies presented in this thesis have arisen from an industry-driven interest to investigate alternatives to conventional fertiliser regimes in temperate fruit orchards, and accompany field trials which have been established as part of a larger project. In this thesis, both model pot-grown studies and field studies have been conducted, with themes related to organic amendments and plant productivity.

1.1 Soil health: An Overview

Soil health in an agricultural context can be referred to as the ability of the soil to perform agricultural functions; these include the enhancement of crop growth and resistance to biotic and abiotic stresses under intensive management and fertiliser inputs (Magdoff, 2001, Singh *et al.*, 2011). Soil health has been further defined as the ability of a soil to act both within the limits of its ecosystem and the external environment to that ecosystem (Lobry de Bruyn and Andrews, 2016).

Parameters often measured to determine the level of soil health include: organic matter content, soil biological quality, soil organic carbon, the relative absence of pests and pathogens, existence of sufficient reserves of nutrients and balance in nutrient ratios. Healthy soil also must have a strong resistance to degradation processes (Magdoff, 2001, Farquharson *et al.*, 2003, Gil *et al.*, 2009a, Gil *et al.*, 2009b, Singh *et al.*, 2011). According to Passmore and Brown (1991), Magdoff (2001) and Farquharson *et al.* (2003), soil degradation can be evaluated through soil erosion rate, and intensive agriculture, tillage practices, soil exposure to drought and rapid wetting (floods, heavy rains and irrigation) could expose the soil to erosion, which can result in lower carbon content of the soil. Soil erosion rate for different parts of the field or between fields can be measured by comparing the vertical distribution of sediment, soil carbon and organic matter (Ritchie and McHenry, 1990, Farquharson *et al.*, 2003). Soil density and the soil capacity to store water are also used to assess soil erosion (Karlen *et al.*, 1997).

1.2 Soil organic matter

Soil characteristics are affected by the management of soil and crops (Magdoff, 2001). Reduced fertility of agricultural soils has resulted in a dramatic increase in the use of soil amendments to improve the quality of agricultural soils. One of the biggest causes of declining soil fertility is depleted organic matter content in the soil associated with poor

agricultural practices (Wells *et al.*, 2000) and environmental conditions such as hotter and drier environment, which are often accompanied by limited water content (Singh *et al.*, 2011). Declining soil organic matter can result from changes in soil physical, chemical and biological characteristics.

1.3 Organic amendments

The addition of organic matter will lead to changes in the accumulation of soil particles and mineral nutrients, as well as changes in the soil's capacity to retain water (Reeves, 1997). Many soils with poor structure require the use of amendments to ameliorate their structure. For instance, adding gypsum into sodic soils will improve their structure and help to avoid soil degradation (Singh *et al.*, 2011). Singh *et al.* (2011) mentioned that organic amendments, especially those with high organic carbon such as biochar and compost, also assist to improve soil structure. Working on a potato field, Wells *et al.* (2000) reported that after one cropping season it was observed that the soil amended by 67 mg ha^{-1} of mixture of organic materials comprised from beef cattle manure and compost had a higher content of organic carbon, cation exchange capacity and aggregate stability.

Organic amendments comprised of plant residues often contain the remains of plant roots, which may contain inoculum of microorganisms such as mycorrhizal fungi and/or nitrogen-fixing bacteria that can indirectly benefit soil health (Magdoff, 1996, Innerebner *et al.*, 2006, Cavagnaro, 2014, Cavagnaro, 2015). Increase in microbial biomass is one of the bio-indicators of improvements in soil health, as soil microbes are involved in the processes of mineralisation of organic material (Sangha *et al.*, 2005).

The addition of compost to agricultural soils has important benefits, including reducing soil acidity (Pocknee and Sumner, 1997, Wells *et al.*, 2000), providing a full range of nutrients to the soil (Tejada *et al.*, 2001, Zheljazkov and Warman, 2004), improving water holding ability (Wells *et al.*, 2000), increasing the beneficial organisms in the soil and reducing plant pathogens (Abawi and Widmer, 2000), and release of humate substances such as humic and fulvic acids (Cavagnaro, 2015). The degree of improvement in soil physical, chemical and biological characteristics can vary depending on the type and source of the organic material used in compost preparation, as well the quantity added.

Organic acids such as humic acid and fulvic acid can benefit soil health by improving the soil's ability to retain moisture, nutrients, soil structure and increasing microbial activity (Ouni *et al.*, 2014). Ouni *et al.* (2014) mentioned that humic acids have the ability to decrease

evaporation in arid, sandy and non-clay soils, as well as enhance the conversion of some mineral nutrients into their plant available forms. Humic substances including humic acids are beneficial to the health of the soil because they influence a number of chemical and physical characteristics of the soil (Zhang *et al.*, 2013, Ouni *et al.*, 2014). Humic substances affect sorption and transport of hydrophobic organic compounds, the stability of the aggregates, the ability of buffering and the complexity of minerals existing in the environment. They also promote biological activity in the soil. Further, humic substances have the ability to sequester adsorbent and chelating, from metals (minerals and pesticides) and organic compounds, by conferring them a key role in the degradability, solubilisation, bioavailability, and exchange and transport of these compounds in soil and water (Ouni *et al.*, 2014). Decomposed organic matter in the soil, comprised of plant and animal waste from degradation and activity of microbes, contains a large portion of these humic acids (Gulser *et al.*, 2010). Research has shown that humic substances released from organic matter decomposition influence soil properties. Zhang *et al.* (2013) reported that humic acid works to reduce the acidity of the soil, which in turn enhances the absorption of essential nutrients for plant growth, especially micronutrients. Ouni *et al.* (2014) reported that humic substances reduce soil pH, Na and EC because of high supplies of Ca, K and Mg. Humus complexes have great ability to adsorb sodium ions (Na^+) that lead to soil salinisation and plant toxicity (Ouni *et al.*, 2014).

In recent times, the use of biochar as an organic soil amendment has received considerable attention. Eyles *et al.* (2015) reported that biochar is considered a carbon sink, and helps to improve a range of soil functions and physical characteristics and also promotes plant growth.

1.4 Biological inoculants

Soil microbes have great importance in maintaining soil fertility and health through cycling of organic and inorganic nutrients. There are many groups of beneficial microorganisms in the rhizosphere including mycorrhizae and bacteria such as nitrogen-fixing bacteria. These groups of organisms form symbiotic associations with plant roots and have been the subject of much research (Jeffries *et al.*, 2003). For instance, plants tend to form symbiotic associations with arbuscular mycorrhizal fungi (AMF) in order to enhance the absorption of nutrients and water from the soil (Brundrett, 2002, Perner *et al.*, 2006, Smith and Read, 2008). In return, AMF plants supply the fungus with photosynthetic carbon that is transported to the soil through fungal hyphae (Smith and Read, 2008). Therefore, AMF hyphae act as a

direct channel between the host C and the soil. This means that AMF directly contribute to the increase of C pools in the soil.

AMF hyphae have an important role in the compilation of soil particles and the formation of macro-aggregates (Andrade *et al.*, 1998, Bethlenfalvay *et al.*, 1999, Miller and Jastrow, 2000). Mycorrhizal fungi produce organic compounds including glomalin and a stable hydrophobic glycoprotein, which is deposited on outer walls of the extraradical AMF hyphae and soil particles (Wright and Upadhyaya, 1998, Wright and Upadhyaya, 1999). Those organic compounds lead to entanglement of soil particles and the formation of macro-aggregates (Miller and Jastrow, 2000). Consequently, decline in AMF propagules, which means lower AMF biomass in the soil, may cause a decline in soil health and soil physical properties, particularly soils that have low porosity. It is well documented that agricultural practices such as intensive agriculture and P fertiliser applications can reduce AMF propagules in the soil (Jeffries *et al.*, 2003, Smith and Read, 2008). Thus, understanding how to choose alternative practices that would increase the propagules of AMF requires attention.

In addition to AMF, plant roots interact with other soil microbes such as bacteria that adapt to live in the root zone. Adapted bacteria in the root zone often work synergistically in association with AMF (Jeffries *et al.*, 2003). Bacteria such as plant-growth-promoting rhizobacteria (PGPR) are important to soil fertility and plant health. Rhizobacteria and rhizofungi influence plant and soil health by production of organic compounds such as plant hormones or vitamins that directly stimulate plant growth (Barea, 2000), and interact with potential phytopathogens (Azcón-Aguilar and Barea, 1997).

The presence of many bacterial species in the rhizosphere is documented to improve soil quality and growth and productivity of crops (Higa and Parr, 1994). The use of beneficial effective microorganisms has become an important part of organic agriculture to improve soil health and crop performance (Yamada and Xu, 2001, Fließbach *et al.*, 2009). Effective microbes (EM) are a commercial culture of up to 80 species of co-existing beneficial microorganisms consisting of lactic acid bacteria, yeasts, photosynthetic bacteria and actinomycetes (Higa and Parr, 1994, Yamada and Xu, 2001). These species have the ability to increase crop productivity by N-fixation, increasing photosynthesis and accelerating decomposition of lignin material in the soil (Bajwa *et al.*, 1999b). Khaliq *et al.* (2006) state that inoculation of soil with EM along with organic or inorganic materials is an effective technique for stimulating the supply and release of nutrients from these materials.

1.5 Why should organic amendments be used?

Organic materials used as organic supplements or amendments include humic substances, seaweeds or seaweed extracts, animal remains such as bone meal and blood, and composted and non-composted organic matter such as plant residues (Eghball and Power, 1999, Imbufe *et al.*, 2005, Curnoe *et al.*, 2006, Nastri *et al.*, 2006, Hargreaves *et al.*, 2008, Mondini *et al.*, 2008), which may contain or be supplemented with microbial species to improve soil heath and crop productivity (Fließbach *et al.*, 2009). Investigations on the impact of organic soil amendments as fertilisers have been evaluated on several crops (Mikkelsen, 2005, Daur and Bakhashwain, 2013, Zhang *et al.*, 2013); however there is a lack of comparative investigations between these applications on the same crop and the different cultivars in Australia in general (Quilty and Cattle, 2011) and in orchard crops. Quilty and Cattle (2011) state that the decline in investigations on organic amendments is due to the high agricultural production requirements, a lack of consistency in the formulation of some products, limited knowledge about the benefits of organic supplements and the lack of unbiased scientific studies for the agricultural potential of these products.

Kibblewhite *et al.* (2008) illustrate the relationship of organic supplements with the health of agricultural soils and their ability to support agricultural economic activity and maintain ecosystem services. Many Australian government institutions have adopted this concept, and worked to establish programs to encourage farmers to improve soil health (McKenzie, 1998, MacEwan, 2007). Quilty and Cattle (2011) reported on a survey conducted to evaluate the problems related to soil health in many Australian cotton farms. Survey results indicated that organic amendments had not been adopted widely by farmers, with approximately 21% using organic amendments on a trial basis and only 13% applying organic amendments regularly.

The reduced availability of P in many agricultural soils is a concern, as it is an essential mineral for food production (Cordell *et al.*, 2009). Cordell *et al.* (2009) state that most of the P that is used to produce food is added by mineral fertilisers; therefore, the use of organic materials for recycling and supplying agricultural soils with mineral nutrients should receive greater attention.

1.6 Agricultural regimes and sustainability

Demand is growing for sustainable agriculture with efficient crop productivity and minimal impacts on ecological factors such as soil fertility (Mäder *et al.*, 2002). Ideally fertile soil promotes plant growth, supports biodiversity and enhances biological activity in the soil.

Many farm managers have adapted or replaced regimes based on conventional management with organic regimes as an alternative to enrich the soil. Rigby and Cáceres (2001) reported that concerns relating to the health and structure of the soil, the rapid rate of nutrient depletion on farms built on a chemical basis (by leaching and volatilisation into the atmosphere), and human health issues have encouraged the early proponents of alternative, biological or organic farming. Despite the above concerns, there is still a lot of discussion revolving around agricultural sustainability under different agricultural regimes, especially to maintain an appropriate renewable level of nutrients over time (Rigby and Cáceres, 2001, Asami *et al.*, 2003).

In typical organic regimes, green manure and animal waste are used to supply plants with necessary nutrients and there is no or minimal use of synthetic fertilisers and pesticides (Liebig and Doran, 1999, Mäder *et al.*, 2002). Crop rotation practices and the use of biological control and tillage to maintain soil productivity are examples of organic regime practices; while in conventional regimes, synthetic fertilisers and pesticides are used (Liebig and Doran, 1999, Mäder *et al.*, 2002). Liebig and Doran (1999) state that all previous studies have focused on long-term effects of organic and conventional regimes, and emphasise primarily the effects related to soil quality. There are insufficient studies on the effects of organic and conventional practices, especially liquid applications, on short-term soil fertility and the growth and development of annual herbaceous crops (Treadwell *et al.*, 2007).

As organic amendments gradually increase soil health there are some important questions that can be asked. Does the addition of organic amendments:

- improve the nutrient content in the soil and crop?
- improve nutrient release in the soil?
- increase microbial activity?
- increase the activity of mycorrhizal fungi in the soil?
- improve crop yield and quality?

1.7 Model crops – sunflower and tree crops

From an agronomic perspective, the provision of nutrients necessary for plant growth is the objective of most trials in relation to the quality and health of the soil. Maintaining soil mineral nutrient levels is important for reducing the impacts of inorganic fertilisers and preserving the environment. Studies in the 1980s focused on producing sunflower crops under intensively irrigated conditions, and explored the possibility of production in limited

water conditions (Unger, 1982, Unger, 1983, Connor *et al.*, 1985). In general, cultivation of sunflower crop requires about 50 - 100 kg ha^{-1} N, 20 - 45 kg ha^{-1} P and 60 - 125 kg ha^{-1} K, and the crop is particularly sensitive to B-deficiency (FAO, 2015). Recently, the Australian Sunflower Association (ASA) and Australian Oilseeds Federation (AOF) stated that land devoted to the cultivation of grains is exposed to the depletion of nutrients, especially N and K (AOF and ASA, 2013), but compared with land planted with wheat, corn and sorghum, sunflower cultivation depletes large amounts of N and K in comparison with the low productivity of this crop. Nutrient depletion in intensive horticultural orchards cultivated with perennial trees such as apples and cherries is also high; however, the degree of depletion differs from orchard to orchard due to differences in management practices (irrigation, size and type of the microbial community in the soil, weed control) and from one region to another due to differences in soil and climate types. Ferree and Warrington (2003) reported the amount of nutrients removed by apple trees during the season (Fig 1.1).

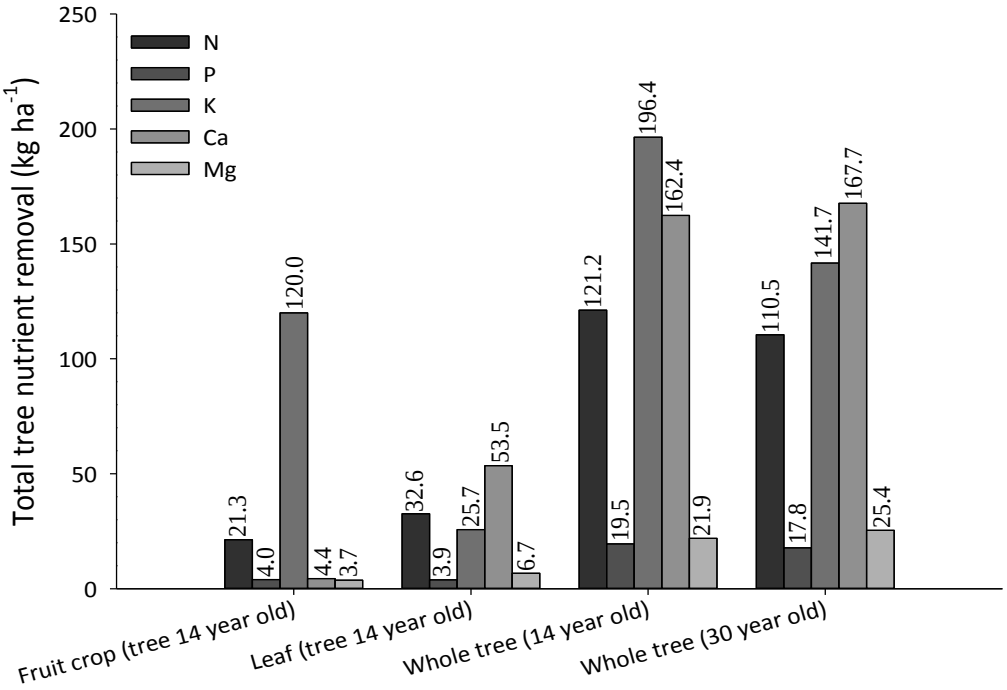

Figure 1.1 The calculated amount of nutrients removed by apple trees from an orchard during the season (whole tree data consisted of top framework, leaf, roots and fruit crop; 14 year old trees were Golden Delicious, 500 trees ha^{-1}, estimated 90 tones fruit ha^{-1}; 30 year old trees were Delicious, 124 trees ha^{-1}, 44.8 tones fruit ha^{-1}), data adapted from Ferree and Warrington (2003).

Research is required to find ways that would provide mineral nutrients during the growing season and at the same time maintain sufficient reserves of nutrients for the following seasons, while maintaining microbial activity. Several studies have compared the possibility

of providing these ratios of nutrients in the soil using organic applications based on plant and animal waste. However, there are not a sufficient number of studies which have evaluated the possibility of liquid organic applications (Treadwell *et al.*, 2007) built on the basis of humic substances and seaweed extracts in conjunction with bio-inoculation (fungi and microbes) to provide the necessary ratios of the above mentioned nutrients, and keep the soil adequately supplied with these nutrients for the following seasons.

1.8 Thesis objectives

Solid soil amendments such as compost are physically difficult to apply on a large-scale, and there is little information on the effects of soil amendments such as humic substances (either granules or soluble granules) and biochar combined with different rates of inorganic and organic fertilisers under greenhouse and orchard conditions; or on the effects of these amendments with fungal and microbial inoculums on the status of nutrients in the soil and plant leaves. Therefore, to evaluate those practices including soil amendments, studies with bio-inoculants and renewable alternative nutrient regimes compared to conventional nutrient regimes were conducted to answer the following questions:

- Are humate additions alone or in conjunction with EM inoculation promoting total plant growth compared with the use of inorganic fertiliser? What are the consequences of high rates of EM inoculation on plant growth? (Chapter 3)
- Are renewable alternative practices providing sufficient reserves of essential nutrients; and in the case of failure of these practices in the provision of adequate reserves of nutrients, can they be integrated with traditional practices for an optimal solution? (Chapter 4)
- What is the impact of these practices in maintaining soil health and promoting plant growth when applied in the short or long term? (Chapter 5 and 6)
- Are these practices able to improve fruit flavour characteristics along with AMF activity in perennial deciduous tree crops? (Chapter 6)
- What are the effects of these practices on colonisation and performance of arbuscular mycorrhizal fungi in the short and long term; and are there consequences for plant growth and the development and improvement of fruit quality? (Chapter 5 and 6)

"Chapter 2" Literature Review

2.1 Introduction and scope

This literature review is focussed on the relationship of soil nutrition and health with plant growth and quality, with particular reference to southern Australian soils and temperate horticultural systems (both annual and perennial). Specifically, the review will outline how plant growth and development is influenced by different management practices (e.g. use of conventional synthetic fertilisers or organic amendments), which alter soil nutrition and microbial composition. This is relevant given a major challenge today is to maintain the quality and quantity of horticultural products while at the same time ensuring sustainable future production. In addition, approaches must be designed to achieve these goals without endangering the environment or public health.

Kennedy and Smith (1995) state that there are many concepts and definitions of sustainability; simply, sustainability is the adoption of agricultural practices that allow maintenance of production capacity for a long time, the safety and quality of products, and preservation of the environment. Currently, the sustainability of horticultural production is at risk due to reduction in the fertility and health of soils, which is typically the result of a range of intensive agricultural practices and intensive use of agricultural lands (Cockroft, 2012). However, this does not mean that these practices should be abandoned or replaced with others, but rather that they should be developed by integrating them with new practices that maintain fertility and health. In this way, the rate at which the productivity of agricultural land is deteriorating may be slowed, or ideally reversed.

In this review, the determinants of soil quality and fertility will be explored in detail and then discussed in terms of different agricultural practices. Conventional practice, in which there is a reliance on synthetic chemicals including pesticides, herbicides and synthetic fertilisers, is typically higher yielding in many horticultural systems than organic practice (Seufert *et al.*, 2012, Tuck *et al.*, 2014). Organic systems prevent the use of synthetic fertilisers, herbicides and pesticides. In these practices the use of cultural controls (such as crop rotation) and organic crop protectants as a strategy to control weeds and pests is more favourable, while animal residues, green manure or compost are used instead of synthetic fertilisers (Seufert *et al.*, 2012, Tuck *et al.*, 2014). A third alternative is an integrated management practice, which uses low inputs of synthetic fertilisers and pesticides and some organic practices. This may present the ideal outcome for sustainable horticulture as, while organic practices have many

benefits, there are some disadvantages for crop nutrition, which will be outlined during this review.

2.2 Soil quality

Glover *et al.* (2000) describe soil quality as the ability of the soil to work within the boundaries of the ecosystem to maintain the biological productivity, the quality of the environment, and to enhance plant and animal health. Fageria (2010) adds that soil quality can be measured by sustainability, productivity, environmental quality, promotion of plant growth, and impact on human nutrition and animal health. Therefore, in the context relevant to this literature review, soil quality can be defined as the ability of the soil to provide crops with essential nutrients in the long term, while maintaining its physical, chemical and biological properties.

Growers often depend on their experience to determine whether soil is more or less fertile (Cotching, 2009). The criteria they use as positive indicators of soil fertility include: soil colour, crop yield, the capacity of soil to hold water, crop growth rate, and presence and abundance of soil macro-fauna (Desbiez *et al.*, 2004, Cotching, 2009, Fleskens and Jorritsma, 2010). Those used as negative indicators are stoniness, difficulty of carrying out horticultural practices such as ploughing, weeding and planting, abundance and diversity of weeds, poor leaf colour, and symptoms of deficiency of elements (Desbiez *et al.*, 2004, Cotching, 2009, Fleskens and Jorritsma, 2010). However, leaf colour cannot be reliably considered as a conclusive sign of poor soil quality unless deficiencies confirmed by tests showing deficiency of a specific element. This element may be present in the soil in abundant quantities, but in a form that is unavailable to the plant. Alternatively, the colour change may be the result of another abiotic or biotic factor, for example viral infection in the plant or a wilt disease which causes clogging of its xylem and phloem vessels (Dawoe *et al.*, 2012).

It is well established that parent materials have the main influence on soil properties (Yost *et al.*, 1982, Schaetzl and Anderson, 2005). For example, in a study of 39 soils derived from various parent materials in south-western Australia, Singh and Gilkes (1992) reported that the content of minor elements (Fe, Cd, Zn, Cu, Mn, Ni, Co) related to the parent materials. Many soil characteristics, such as aggregation of soil particles, soil colour, the amount of humus and salt content are gained from parent material (McKenzie *et al.*, 2004, Schaetzl and Anderson, 2005). As soils develop, these characteristics become more influenced by climatic factors as erosion and weathering processes can destroy the core layer that formed from the

parent material and form new layers (Schaetzl and Anderson, 2005). This in turn changes soil quality. Leaching processes can reduce the concentration of organic material, as well as alter the proportion of salt and soil acidity, especially in the upper layers of soil (Schaetzl and Anderson, 2005). A range of acids in the rhizosphere produced from degraded roots and organisms can cause oxidation and a considerable loss of organic materials (Tisdall and Oades, 1982, Oades, 1984). The change in the physical properties of organic matter makes it unstable and prone to loss by quick wetting. However, many of the characteristics of the parent material remain stable, and will continue to exert their effects on the soil for long periods of time (Schaetzl and Anderson, 2005).

In general, soil consists of clastic particles (mineral materials), organic matter in various stages of decomposition, living organisms, water or ice and gases within the pores between soil particles (Schaetzl and Anderson, 2005). These components are clustered in different proportions from one soil to another. Figure 2.1 illustrates volumetric soil composition under normal circumstances, but all of these proportions differ from one soil to the other. The proportion of organic material range from 1% to 8%, mineral proportions are often 40-48% in most inorganic soils, the amount of water can be increased and the volume of air between the particles varies from one soil to another (Schaetzl and Anderson, 2005). Clastic mineral particles in the soil are divided into fine particles (less than 2 mm diameter) and coarse particles (bigger than 2 mm diameter). The division of soil particles on the basis of the size of the granules forms different soil types. The arrangement of these mineral clastic particles gives the so-called soil structure, which forms the skeleton of the soil.

The aggregation of soil particles not only constitutes soil structure, but affects the bulk density of the soil (Schaetzl and Anderson, 2005). The main determinant of the increase or decrease in soil bulk density is the presence of voids and pores. Living organisms in the soil, especially macroscopic soil fauna, such as termites, worms and many other forms of fauna are one of the factors that create or maintain high soil porosity. Organic matter in the soil decreases bulk density; some organic soils have bulk density less than 1.0 g cm^{-3} because the organic matter attracts soil fauna that make the pores. Compacted soils and those that have low organic matter can have a bulk density as high as 2.3 g cm^{-3} (Manrique and Jones, 1991, Schaetzl and Anderson, 2005). Although silt and clay soils have lots of micro-pores they tend to have high bulk density, because the clay and silt can fill large voids.

Soil structure can be degraded by horticultural practices. For example, Tennant *et al.* (1992) state that there are problems in most of the Western Australian soils that have been subject to horticultural practices. These problems include widespread wind and water erosion, acidification, degradation, non-wetting, waterlogging in medium and high rainfall areas, and the spread of secondary salinisation. Hardie and Cotching (2009) describe the impact of intensive lettuce production on a Chromosol soil in southern Tasmania. Soils are cropped two to three times and rotary hoed up to 15 times a year; annual irrigation is 5-7 ML ha^{-1}. These practices have resulted in a decline in soil structure, loss of soil organic carbon, bed collapse and surface crusting, leading to poor efficiency of irrigation, reduction of infiltration rate, erosion and runoff.

2.3 Soil fertility

A simple definition of soil fertility is the ability of the soil to provide the appropriate level of plant nutrients required to increase yield and improve crop quality, while at the same time maintaining sustainability. Reganold *et al.* (2001) describe soil fertility as the soil's ability to maintain biological productivity, environmental quality, and promote plant and animal health. Sparrow *et al.* (2013) state that changes in land management are usually accompanied by changes in soil properties. For example, a rapid loss of soil carbon occurs when land is tilled for the first time (Bowman *et al.*, 1990). Changes in soil properties due to land management could lead to a significant change in an "existing equilibrium" (Sparrow *et al.*, 2013) which means a change in soil fertility.

Along with other important nutrients such as phosphorous and potassium (K), nitrogen (N) is an essential element for plant growth. In addition these nutrients need to be in a form that is available to the plant. There is a belief that the addition of large amounts of N through chemical applications will solve deficiency problems. Several studies have demonstrated that even when plants with symbiotic fixation of N are present, N cannot be produced in the same quantities as those which can be added artificially (Chikowo *et al.*, 2004, Havlin *et al.*, 2005). However, practices used to correct the deficiency of a specific element, such as adding fertiliser compounds containing the element, may result in nutritional imbalances in plant tissue, such as change in N status in plant flowers that received higher rates of N fertilisation. This, in turn, can lead to a lowering of the plant's resistance to insect pests (Altieri and Nicholls, 2003). This phenomenon may be explained as either a physiological change in plant cells making them more susceptible to harmful microbes, or a change in the status of elements in plant tissue which makes the plant more palatable to herbivorous animals.

Hence it becomes obvious that soil fertility relies on the total amount of nutrient minerals that are available to the plant rather than the total amount of elements that are fixed in the soil, as these may not be accessible to the roots of the plant. These nutrients are necessary, especially nutrients that are not present in animal manure, or are present, but in quantities that are insufficient to meet plant requirements.

2.4 Determinants of quality and fertility of the soil

2.4.1 Living organisms in the soil

What occurs under the soil's surface is of critical importance to soil fertility and therefore, by definition, to maintenance of sustainability; and soil biota is essential for nutrient cycling and plant growth. While it is well known that many soil borne organisms can be harmful to plants as pathogens (Agrios, 2005), symbiotic associations between beneficial microbes (e.g. mycorrhizal fungi) and plant roots can provide many advantages to plant growth and health.

Feeding plants with synthetic nutrients in conventional agriculture and horticulture practices has been prevalent, mainly because the total quantity of elements fixed in the soil has been the main measure of soil fertility. Focus on the positive effects of the coexistence between plants and soil microbes has been changed in the prevailing long-held belief that NPK should be used as a primary plant food (Pimentel *et al.*, 2005, Birkhofer *et al.*, 2008). This mind-shift has subsequently opened the door to the use of microbes as commercial bio-fertilisers (Egerton-Warburton and Allen, 2000, Egerton-Warburton *et al.*, 2001, Wu *et al.*, 2005, Adesemoye *et al.*, 2008, Barrow, 2012). However, this does not necessarily mean that conventional applications, such as NPK, should be dispensed with altogether.

2.4.1.1 Bacteria

Soil microbes play major roles in ecosystems and they affect a large number of important ecosystem processes, including the acquisition of nutrients, N- and C-cycling (Van Der Heijden *et al.*, 2008), and soil formation (Rillig and Mummey, 2006). In agricultural systems, Van Der Heijden *et al.* (2008) reported that soil microbes have a significant influence on plant productivity through two main mechanisms. The first mechanism is through the direct influence on plant growth by soil micro-organisms associated with roots forming beneficial mutualistic relationships. The second mechanism is through an indirect effect, via the activity of free-living microbes that change the rates of mineral nutrient supply. Van Der Heijden *et al.* (2008) mentioned that a large group of soil microbes are symbiotically associated with

plant roots and these improve plant productivity by providing a limited level of mineral nutrients for plants.

Plant growth promoting bacteria

Plant-growth-promoting-rhizobacteria (PGPR) can be either symbiotic or free-living and their presence is associated with higher levels of available N in soils (Hayat *et al.*, 2010). The most well-known symbiotic PGPR associations are those between leguminous plants and N-fixing bacteria in the soil, which convert atmospheric-N into a plant usable form (Singh *et al.*, 2010, Bhattacharyya and Jha, 2012). Nitrogen fixing bacteria contribute to plant productivity in tropical savannah and some tropical forests and grasslands which are dominated by legume plants (Van Der Heijden *et al.*, 2008). However, the legumes can also make a contribution to horticultural and field crops. Granatstein *et al.* (2012) state N is an essential nutrient needed in most Pacific Northwest orchards in the USA, especially in orchards established on an organic basis. Planting legumes rather than grass in the alleys of fruit orchards can provide a portion of the orchard N required from N fixation (Granatstein *et al.*, 2012, Oliveira *et al.*, 2016). Granatstein *et al.* (2012) found that the N concentration in the tissues of legumes (alfalfa (*Medicago sativa*) 4.06%, Jumbo Ladino white clover (*Trifolium repens*) 3.77% and birdsfoot trefoil (*Lotus corniculatus*) 3.36), planted in a Western Australian apple orchard, exceeded its concentration in grass tissues (grass 2.15 and non-legume 2.32). These authors concluded that, for greater beneficial effect, leguminous crops could be mowed or mowed and tilled into the tree row.

To mitigate the potential environmental damage as a result of some horticultural practices, the focus has turned to the role played by micro-organisms in the soil to increase the efficiency of water and nutrient use and uptake capacity (Armada *et al.*, 2014). Many studies have been conducted to test the efficacy of PGPR strains on the growth and yield of fruit and vegetable crops, and flower and ornamental plants (Ruzzi and Aroca, 2015). For example, *Bacillus* sp. strain M3[a], OSU-142[a], *Microbacterium* sp. strain FS01[e] and *Pseudomonas* sp. strain BA-8[d] (alone or in combinations) were applied by root-dipping (109 CFU mL^{-1}) prior to planting and treated trees showed increased shoot length, fruit weight, cumulative yield, and shoot diameter in apple cv. Stark Spur Golden and Granny Smith (Aslantaş *et al.*, 2007, Karlidag *et al.*, 2007). When *Pseudomonas* sp. strain BA-8[d] (alone or in combinations) was applied in the field, it led to a significant increase in yield per unit trunk cross-sectional area, improved plant growth, shoot length and fruit weight in sweet cherry (Esitken *et al.*, 2006). Göre and Altin (2006) observed that when *Pseudomonas fluorescens* strain 51[d] was applied

as a soil drench (10^{11} CFU per plant) in a Chrysanthemum pot trial under glasshouse conditions, it increased leaf surface area, number of flowers and plant height.

Bacterial biodiversity and its relationship to soil characteristics

According to Torsvik and Øvreås (2002), the stress and disturbance that usually occur in orchards influence the productivity of these orchards as a result of changes that occur to the distribution of microbial communities. Blankinship *et al*. (2014) reported that microbes break down soil organic matter. An understanding of how their presence and diversity is related to soil (and in turn influences soil characteristics) is important in horticultural production systems.

A number of studies confirm the effect of soil structure and spatial isolation on the activity and diversity of microbial communities (Sessitsch *et al.*, 2001, Torsvik and Øvreås, 2002). The spatial distribution analysis of bacteria in levels of micro-habitat showed that, in soils that have been subjected to various fertilisation treatments, more than 80% of the bacteria were present in the micro pores in the stable soil micro-aggregates (2-20 µm) (Torsvik and Øvreås, 2002). Micro pores provide the most favourable conditions for the growth of microbes, such as the appropriate moisture, the volume of appropriate gas to these habitats and protection from predation (Torsvik and Øvreås, 2002). Sessitsch *et al.* (2001) believe that soil particle size (which is a major factor in determining the nature of soils such as clay, sand and silt) has the largest effect on the microbial diversity and size of microbial biomass, compared to other factors such as soil pH and organic inputs. Results of Sessitsch *et al.* (2001) showed that microbial diversity in small soil particles (silt and clay), especially species that belong to the division of *Holophaga*/*Acidobacterium* (22% classified as *α-Proteobacteria*, 16% high-GC gram-positives, 10% *Cytophaga/Flexibacter/Bacteroides*, or *β-Proteobacteria* (8 and 4%)) and *Prosthecobacter* group, was higher compared to the microbial diversity in large soil particles. In contrast, microbial diversity associated with large soil particles (sand) was dominated by bacteria belonging to the α-proteobacteria with few members of *Holophaga*/*Acidobacterium* division (Sessitsch *et al.*, 2001).

Other investigations have found that the amount and type of available organic material have a greater impact on the diversity and abundance of microbes in the soil, rather than the impact of the size of the soil particles (De Fede *et al.*, 2001, Grayston *et al.*, 2001). Smit *et al.* (2001) studied relationships between the abundance of microbial communities and nutritional status of the soil for five divisions of bacteria through the distribution of 16S rDNA sequences.

Their results indicated that soil with a high content of readily available nutrients exhibited positive selection for α- and γ-proteobacteria, this being evidence of r-selection (selection for bacteria with high growth rates). In soil with a low content of nutrients (or high content of recalcitrant substrates), the proportion of *Acidobacterium* increased, this also being evidence of k-selection, that is selection for bacteria with possibly lower growth but higher capacity to compete on substrates. The proportion between the number of *proteobacteria* and *Acidobacterium* could be evidence of the nutritional status of soils (McCaig *et al.*, 2001, Smit *et al.*, 2001).

Torsvik and Øvreås (2002) reported that competitive interactions between soil microorganisms are a key factor in controlling the microbial community structure and diversity. Soil structure and water systems affect the competitive interactions by causing spatial isolation within local communities. Soil with high spatial isolation has been found to exhibit higher diversity of microbes (Torsvik and Øvreås, 2002). High temperatures with high spatial isolation can affect the microbial diversity in the soil, by causing high heterogeneity in carbon resources (Torsvik and Øvreås, 2002). Smit *et al.* (2001) noted that the change in the bacterial biomass during the season is not great. However, when culturing techniques were used for field soils to assess the composition of the microbial community through molecular fingerprinting techniques, large differences on the microbial community between winter and summer were detected. While the content of the cultural medium of fast-growing bacteria was highest in summer and lowest in winter; the highest level of species richness was observed in spring and autumn (Smit *et al.*, 2001). The increase in the diversity and activity of microbial communities during the spring may be due to fertilisation (in the spring) and degradation of plant debris after harvest in the autumn.

2.4.1.2 Fungi

Improving the physical and chemical properties of soil is not confined to adding organic and chemical fertilisers to provide the necessary nutrients for plant growth. A major role is also played by the presence and activity of both naturally occurring and commercially added beneficial fungi. These can influence the physical and chemical properties of the soil by releasing nutrients that were fixed in the soil. Fungi are a diverse group of organisms with a wide range of forms, from microscopic single-cell yeasts to large macro-fungi such as mushrooms and the giant puff-ball (Kendrick, 2000, Bridge and Spooner, 2001). The life cycle of most fungi is associated directly or indirectly with a soil environment, for it is in the soil that they spend at least part of their life in the form of either spores or mycelium.

While several soil fungi can provide direct or indirect benefits to plant growth, in this literature review the focus is restricted to mycorrhizae. In an 1885 study examining the nutritional dependence of trees from the family Cupuliferae (now referred to as Fagaceae) on root symbiosis with below-ground fungi, Frank (2005) derived the term 'mycorrhiza', meaning fungus-root to describe the fungal mantle enclosing the roots. Frank described this symbiosis between the roots and fungal mycelium as performing the function of a "wet nurse", providing the entire nourishment of the tree from the soil. As shown in Table 1, mycorrhizal fungi are classified into seven main groups (arbuscular, arbutoid, ectendo, ecto, ericoid, monotropoid and orchid mycorrhiza) based on the common attribute of being "*largely aseptate endophytes in the Glomeromycota and those formed by septate fungi in the Ascomycetes and Basidiomycetes*" (Siddiqui *et al.*, 2008, Smith and Read, 2008). Arbuscular and ecto-mycorrhizas are the most abundant and widespread in the soil (Allen *et al.*, 2003, Siddiqui *et al.*, 2008). Siddiqui *et al.* (2008) and Fulton (2011) reported that more that 80% of mycorrhizal plants are associated with arbuscular mycorrhizae.

Table 2.1 The attributes of important mycorrhizal types. Entries in brackets show rare cases. Table adapted from Smith and Read (2008).

Type of mycorrhiza	Arbuscular mycorrhiza	Ecto - mycorrhiza	Ectendo - mycorrhiza	Arbutoid mycorrhiza	Monotropoid mycorrhiza	Ericoid mycorrhiza	Orchid mycorrhiza
Plant taxa	Bryophyta Pterido Gymnospermae Angiospermae	Gymnospermae Angiospermae	Gymnospermae Angiospermae	Ericales	Monotropoideae	Ericales Bryophyta	Orchidales
Fungal taxa	Glomero	Basidio/Asco (Gymno)	Basidio/Asco	Basidio	Basidio	Asco	Basidio
Fungi septate	No	Yes	Yes	Yes	Yes	Yes	Yes
aseptate	Yes	No	No	No	No	No	No
Intracellular colonisation	Yes	No	Yes	Yes	Yes	Yes	Yes
Fungal mantle	No	Yes	Yes/No	Yes/No	Yes	No	No
Hartig net	Yes	Yes	Yes	Yes	Yes	No	No
Achlorophylly	No (Yes)	No	No	No	Yes	No	Yes

Colonisation of roots by mycorrhizae

Arbuscular mycorrhizal fungi (AMF) colonise plant roots via three major sources of inoculum in the soil: spores, colonised root fragments and hyphae (Kendrick, 2000, Smith and Read, 2008). For some time it was assumed that the spores were the most important source of colonisation; however, this depends on the species of AMF. Spores in soil differ in size, age and dormancy; big spores with thick resistant walls and many nuclei survive for longer compared to smaller spores (Kendrick, 2000, Smith and Read, 2008). In some species,

colonisation via spores can be poor or occurs somewhat slowly as a result of differences in the vitality of spores and the thickness of the walls (Kendrick, 2000, Smith and Read, 2008). Arbuscular mycorrhizae exhibit varying capabilities in colonising roots according to the different sources of inoculum. Klironomos and Hart (2002) examined the capabilities of three kinds of inoculum (hyphal fragments, fresh root fragments colonised by hyphae, and spores) of eight AMF to colonise *Allium porrum* roots. Results showed that the colonisation of roots by *Acaulospora* and *Glomus* spp. occurred from all three sources of the inoculum, while for *Gigaspora* and *Scutellospora* spp. the colonisation of roots was fully reliant on spores.

Even in the presence of large numbers of spores for various species of fungi, plant roots grow in environments that are composed of a complex underground network of mycelium and roots (Smith and Read, 2008). Smith and Read (2008) reported that even if plant roots had weak growth under different environmental conditions during the growing seasons, there is evidence that the mycelial networks remain intact under frozen or dry soil conditions, which may be causing a lack of root growth, and this has a major role in the colonisation of new plant generations. Olsson et al. (2002) stated that the survival of mycelium networks means availability of a continuous source of organic carbon (org-C) in the soil.

It can be concluded that disrupting the mycelium networks, for example by soil tillage, can lead to a significant reduction in colonisation potential of plant roots and a decline in the absorption of nutrients by plants. Therefore, spores remain important sources of inoculum when the mycelium becomes disrupted. Even if spores are not the most common source for colonisation, they are an essential resource for the survival of many AMF species.

Mycorrhizal symbiosis and plant growth

There is a growing understanding about the plants that rely on, or associate with, mycorrhizal fungi and the consequence of symbiotic interactions, ranging from mutual benefit to parasitism and the impact of environmental conditions on the status of that relationship (Johnson *et al.*, 1997, Jones and Smith, 2004, Moore *et al.*, 2015). Moore *et al.* (2015) described mycorrhizal fungi as having a parasitic or symbiotic relationship while Siddiqui *et al.* (2008) describes it as a symbiotic relationship with plant roots similar to root nodule bacteria. Smith and Read (2008) stated that there are a few plants such as *Zea* and *Allium* which synthesise a yellow pigment when colonised by AMF, but this is not a sufficient reason to diagnose whether AMF are generally useful for plant growth. They report that several investigations have demonstrated that colonisation is often accompanied by a

substantial stimulation of growth. For example, Yan *et al.* (2012) found that the inoculation of AMF increased growth of cucumber seedlings as well as leaf chlorophyll content and soluble sugar content. Working on field grown sunflower plants, Chandrashekara *et al.* (1995) observed that total P uptake and total dry biomass were significantly higher in inoculated plants compared to non-inoculated plants at later stages; however, AMF inoculation during the vegetative stage had no effect on total P uptake and total dry biomass. In a pot trial with apple seedlings Miller *et al.* (1985a) found that plant height increased at week 11 with five out of seven species of AMF; three species increased stem diameter with low P soil, while two species increased plant height with high P soil at week 3, 5 and 7, and stem diameter at week 11. These authors also observed that two species increased stem P and one increased root P at low P soil, while only two species increased leaf P at high P soil.

The acquisition strategies of nutrients for more than 80% of land plants involve the mutualistic relationship with AMF (Smith and Read, 2008). Several investigations have found that the presence of AMF has a greater impact on plant growth in soils with low or imbalanced nutritional status, especially when the level of available P is low (Janos, 1980, Miller *et al.*, 1985a, Marschner and Marschner, 2012, Demir *et al.*, 2015).

The presence of AMF enhances plant growth by several mechanisms. It is clear from several studies (Hattingh *et al.*, 1973, Ames *et al.*, 1983, George *et al.*, 1992, Smith *et al.*, 2000, Wang *et al.*, 2002) that AMF have the ability to absorb, transport and deliver nutrients from the soil to the host plant under a range of environmental conditions. Mycorrhizal colonisation can also enhance the absorption of iron (Fe) (Caris *et al.*, 1998), sulphur (S) (Rhodes and Gerdemann, 1978, Allen and Shachar-Hill, 2009), and zinc (Zn) (Bürkert and Robson, 1994, Jansa *et al.*, 2003). It was also noted that an increase in copper (Cu) absorption accompanies mycorrhizal colonisation (Li *et al.*, 1991, Lee and George, 2005).

The symbiosis with AMF provides protection for host plants from the risk of drought stress; the symbiosis often leads to a change in water relations within the plant, the consequences of this change is the hydration of plant tissue (Augé, 2001, Ruiz-Lozano, 2003, Augé, 2004, Porcel and Ruiz-Lozano, 2004). Ruiz-Lozano (2003) also adds that mycorrhizal symbiosis can protect the host plant from drought risks through a combination of physical, cellular and nutritional effects. Arbuscular mycorrhizal fungi absorb water via their hyphae and deliver it to the host plant (Hardie, 1985, Ruiz-Lozano and Azcón, 1995, Marulanda *et al.*, 2003), promoting plant gas exchange and water use efficiency (Ruiz-Lozano *et al.*, 1995a, Ruiz-Lozano *et al.*, 1995b, Goicoechea *et al.*, 1997, Green *et al.*, 1998), and improved osmotic

adjustment of mycorrhizal plants (Ruiz-Lozano *et al.*, 1995a, Augé, 2001, Kubikova *et al.*, 2001). According to Augé (2001), AMF have the ability to improve soil water retention characteristics, but in later investigations Augé *et al.* (2004) stated that the role of mycorrhiza in improving soil water retention characteristics is still doubtful. Supporting to Augé *et al.* (2004), Rillig *et al.* (2010) observed that there were no significant differences in water leaching from the inoculated and non-inoculated soil by AMF.

Mycorrhizal symbiosis and nutrient transfer into plants

Koltai and Kapulnik (2014) illustrate that in non-mycorrhizal plants, nutrients can be transferred from the soil solution into the cytoplasm via the root epidermal cells. Non-mycorrhizal plants can increase the epidermal surfaces that allow the capture of nutrients through the formation of root hairs. Nutrients are radially transported through root cortical symplasm to the central cylinder. Nutrients may transfer through the root apoplast before being captured into the cytoplasm at the endodermis (van Iren and Boers-van der Sluijs, 1980). In mycorrhizal plants nutrients can be captured into the root cortex through the intraradical AMF mycelium (Koltai and Kapulnik, 2014). The AMF hyphae release nutrients in the soil solution; nutrients can be moved into the plant cytoplasm via membranes adjacent to AMF hyphal structures (Koltai and Kapulnik, 2014).

The contribution of AMF symbiosis in the absorption of nutrients rarely occurs under conditions of high P fertilisation (due to a decline in AMF colonisation) and low N availability (Koltai and Kapulnik, 2014). Other mineral nutrients do not normally have a significant impact on the development of the AMF symbiosis, unless they are at a toxic level (Koltai and Kapulnik, 2014). The increase in the availability of Fe (Caris *et al.*, 1998), phosphorus (P) (Maldonado-Mendoza *et al.*, 2001), Zn (Chen *et al.*, 2003), N (Azcón *et al.*, 2008) and S (Allen and Shachar-Hill, 2009) to plant roots has been exhibited to reduce symbiotic transfer of these nutrients, regardless of intra- or extra radical AMF development.

Mycorrhizal existence and sustainable agriculture

In order to improve crop production in low fertility soils, chemical fertilisers, organic materials and soil management techniques such as planting legumes or fallow are used (Siddiqui *et al.*, 2008). To minimise external inputs and maximise their efficiency, Siddiqui *et al.* (2008) suggested that use should be directed to biological methods such as microbial and fungal inoculations to improve soil conditions, promote the biological activity of the soil and enhance the nutrient cycle.

At present, sustainable farming systems strive to utilise natural fertilisation sources to achieve satisfactory levels of productivity and quality of outputs with less negative environmental impacts (Harrier and Watson, 2004). To achieve the latter, earthworms and micro-symbionts were used as soil biota management (Siddiqui *et al.*, 2008). These soil organisms probably represent approximately 90% of the biological activity in the soil, and this contributes to nutrient cycling, improvement of soil fertility and promotes the symbiotic processes in the rhizosphere (Siddiqui *et al.*, 2008). Taking into account the role played by earthworms and micro-symbionts, fungal symbiosis is mainly responsible for transferring and delivering the most nutrients cycled in the soil into the plant (Duhamel and Vandenkoornhuyse, 2013). Therefore, avoiding agricultural management practices that would reduce biodiversity (tillage, the use of agricultural pesticides and conventional agricultural practices) and finding optimal methods could increase the productivity of biological systems (Duhamel and Vandenkoornhuyse, 2013). Van der Heijden *et al.* (1998) showed that ecosystem productivity was linked to the amount of activity and diversity of fungal symbionts, especially AMF. To achieve intensive agriculture with environmental sustainability, it is important to protect the ecological functions undertaken by AMF, in addition to the neo-domestication (modifying plants genetically to obtain better and more beneficial qualities) of plants to withstand the environmental stresses that mycorrhizal symbionts cannot adequately address.

2.4.1.3 Macro-organisms

Activity of larger organisms (macro-fauna) in the soil, such as ants, earthworms and termites, also contribute both directly or indirectly, to improving soil fertility. Earthworms burrow, making holes which aid in improving soil aeration and so increasing microbial activity. Ants carry soil particles from one place to another, resulting in improvements in the structure of the soil particles. According to Swift and Bignell (2001), soil fauna such as protozoans, nematodes and some mites may enhance soil fertility by providing an environment that encourages microbial growth within their gut, or by exuding into the rhizosphere certain compounds, such as excrement, which can function as niches for some microbes. However, some macro-fauna can also ingest microorganisms or microbial metabolites. Thus, maintaining these organisms by employing sound farming practices, both organic and integrated systems, can enhance soil fertility. The effects of these agricultural practices on the activity of soil fauna (macro-fauna) will not be dealt with in this literature review.

2.5 The role of nutrients

Many elements exist in the environment either naturally or as a result of being added commercially. All living and non-living things on the earth's surface, including plants and organisms such as bacteria and fungi, contain these elements in the atomic structure of their cells (Epstein, 1971). Plants need nutrient elements, such as N, S and P, to manufacture proteins and nucleic acids (Havlin *et al.*, 2005, Steward, 2012). Other elements such as magnesium (Mg) and micro-nutrients, with the exception of chlorine, play a role in the formation of organic structures and enzymes (Marschner, 1995, Marschner and Marschner, 2012). An element is considered essential when symptoms of its deficiency, such as stunted growth, or purpling or yellowing of leaves, are evident on the plant (Epstein, 1971). The plant-available level of these nutrients in the soil is not only related to the quantities in which they are added, but also to factors that affect their availability, such as soil moisture, soil pH and the concentration of soil microbes, all of which are affected by the types of agricultural management practices used (Havlin *et al.*, 2005, Marschner and Marschner, 2012, Srivastava, 2012).

Failure of plants to absorb these nutrients in sufficient quantity leads to mineral deficiency in their tissues. This, in turn, has an adverse effect on plant growth and development. Plants may fail to absorb nutrients for several reasons, for example diffusion and mass flow of ions of low-molecular-weight nutrients could be restricted at the external surface of roots in the absence of root hair biomass (Marschner and Marschner, 2012). The main barrier for young roots is the endodermis and the innermost layer of the cortex cells, and while the walls of the endodermal cells of old roots create an effective barrier against the movement of microbes, it in turn prevents the flow and diffusion of ions of low-molecular-weight nutrients dissolved in the soil solution (Marschner and Marschner, 2012). Other soil related factors that may hinder or reduce the absorption of nutrients include pH, interactions between ions in the rhizosphere, competition between ions, cation:anion relationships and water relations (Havlin *et al.*, 2005, Marschner and Marschner, 2012, Steward, 2012).

Nitrogen, P and K are among the macro and essential mineral nutrients for plant growth, and in horticultural systems these are most often supplied by fertilisers or amendments. Precision fertilisation needs to first determine the concentration of these nutrients in the soil, the needs of the crops and also take into account factors affecting their availability in the soil. Supplementation with mineral elements can play a key role in maintaining soil fertility and crop productivity (Aslantas *et al.*, 2010). Thus, to improve soil fertility and crop productivity,

both mineral element supplements and organic material should be used. The former will supply plants with available nutrients quickly without the soil's physical properties first having to be improved, which can be a lengthy process. In the long term, organic additives can be used to improve the physical characteristics of the soil and promote plant growth (Reganold *et al.*, 2001, Adesemoye *et al.*, 2008).

2.5.1 Nitrogen (N)

Nitrogen is a vital nutrient and key determinant of yield and quality in crops. According to several authors (Tisdale *et al.*, 1993, Havlin *et al.*, 2005, Foyer and Zhang, 2011, Polacco and Todd, 2011), its presence in the environment is the key to achieving sustainable crop growth. The atmosphere contains elemental di-nitrogen (N_2), and other gases which are a combination of N and other elements: nitrogen dioxide, nitric oxide, ammonia and nitrous oxide. In fact, N_2 constitutes about 78% of all atmospheric gases (Havlin *et al.*, 2005). In spite of it being present in this seemingly large quantity, a great deal of energy is required to convert N_2 into a form that is useful to plants; however, plants by themselves do not have the ability to do this. Nitrogen has negative environmental impacts when being interrelated with other greenhouse gases (N_2O and oxides of NO_x in agricultural soils' contribution) (Mosier *et al.*, 1998, Vance, 2001, Marschner and Marschner, 2012), including climate change, acidification of soil, eutrophication and threats to the richness and evenness of animal biodiversity in the soil (Polacco and Todd, 2011), as well as human health risks from the consumption of foods with a high nitrate (NO_3^-) content. For instance, Vance (2001) reported that high concentrations of nitrates (exceeding 10 mg NO_3^--N L^{-1}) in drinking water from N-fertiliser has been implicated in methemoglobin anaemia in young children and infants. Highly nitrogenous run-off water from agricultural soil may cause ecological problems such as eutrophication of inland lakes, coastal waters and rivers (Vance, 2001, Foyer and Zhang, 2011). Therefore, appropriate use of N in soils is important.

2.5.1.1 Nitrogen and plant growth

Nitrogen is the largest element needed by plants after carbon, with total plant biomass comprising from 1 – 6% of N (Jones, 2012, Marschner and Marschner, 2012). Plants need N for forming organic compounds, protein and enzymes, as well as creating nucleotides (Foyer and Zhang, 2011, Polacco and Todd, 2011, Kumar and Sharma, 2013). Plants also require it for the absorption of other nutrients (Havlin *et al.*, 2005). The absorption rate of NO_3^- is usually high, resulting in an increase in rhizosphere pH. When plants uptake high levels of

NO_3^-, an increase occurs in OH^- and HCO_3^- anions, and organic anions, transport out of cells, and an increase in cation uptake K^+, Ca^{+2}, Mg^{+2} (Havlin *et al.*, 2005). In contrast an increase in ammonium (NH_4^+) decreases Ca^{+2}, Mg^{+2} and K^+ uptake and increases $H_2PO_4^-$, because high NH_4^+ uptake causes a decrease in rhizosphere pH (Havlin *et al.*, 2005). Therefore, since plants require more N than any other mineral element, its availability determines the productivity both of natural and agricultural ecosystems.

Nitrogen is a nutrient that is mobile within plants. A deficiency of N can occur as a result of poor absorption, although it may be present in abundance in the environment. Symptoms of N deficiency are usually first evident in older leaves (Havlin *et al.*, 2005, Lyle *et al.*, 2006, Foyer and Zhang, 2011). On the other hand, the presence of high amounts of N in plants leads to lush growth, and production of thin-leaved foliage which is more susceptible to damage from drought, frost and attack by pathogens (Lyle *et al.*, 2006). To compensate for a lack of N, N-rich fertiliser can be used in conjunction with optimal methods of reducing wastage of N in the soil, such as retention of suitable amounts of organic matter in the soil. Berger *et al.* (2002) reported that the soil's ability to store N depends on the amount of organic matter remaining in the soil, where it was noted that a large portion of the N in terrestrial ecosystems has been found in organic materials. The decomposition of carbon (C) in mineral soils reduces the C/N ratio and eventually limits N retention (Berger *et al.*, 2002). The wastage of N (Marschner and Marschner, 2012, Havlin *et al.*, 2014) can also be minimised by avoiding horticultural practices that can cause an imbalance in the microbial biomass balance (e.g. tillage, pesticides and the use of chemical fertilisers). Marschner (1995) states that under anaerobic conditions, some bacteria utilise NO_3^- as a recipient of electrons (a process called nitrate respiration) which leads to production of N gases (N_2, N_2O and NO_X). This process causes great losses of N from the soil by denitrification (Marschner, 1995).

2.5.1.2 Mechanisms for obtaining nitrogen from the environment

Most organisms in the environment, including higher plants, cannot directly access the abundant reservoir of existing atmospheric nitrogen (N_2) because the two N atoms share an triple covalent bond (N≡N) which is exceptionally stable (Foyer and Zhang, 2011). To break this bond to form compounds such as ammonia (NH_3) or NO_3^- requires an enormous amount of energy. Despite this, such chemical reactions do occur, either naturally or during industrial processes, and are known as N fixation.

Havlin *et al.* (2005) state that all N sources used by plants are originally in the N_2 form and it is later converted from this gaseous form to other forms such as NO_3^- and/or NH_4^+ that are then available to plants. Most plants absorb N in the form of NO_3^-, and these forms of N are readily available in alkaline soil. Nitrates are formed from N_2 by either free-living bacteria or symbiotic bacteria which form relationships with roots of a small number of leguminous plant species such as beans (*Phaseolus vulgaris* L.), clover (*Trifolium repens* L.), Acacia (*Robinia pseudo-acacia* L.), peas (*Pisum sativum* L.) and peanuts (*Arachis hypogaea*) (Lyle *et al.*, 2006). Di-nitrogen can be metabolized to forms such as NO_3^- or NH_4^+ ions by:

1. Fixation by micro-organisms (bacteria such *Rhizobium, Bradyrhizobium*) that live symbiotically on legume roots.
2. Fixation by free-living or non-symbiotic soil microorganisms (*Archaea*).
3. Fixation as N oxides by electrical discharges.
4. Fixation of NH_3 and NO_3^- by the manufacture of synthetic N fertilisers.

Nitrate production involves two enzyme-catalysed reactions which occur in the roots and/or leaves, depending on the plant species. Both of these reactions occur in stages because plants cannot store nitrite (NO_2^-) in their tissues (Tisdale *et al.*, 1993, Havlin *et al.*, 2005, Polacco and Todd, 2011). Foyer and Zhang (2011) reported that NO_3^- is more mobile than NH_4^+ in most soils. Havlin *et al.* (2005) further state that NO_3^- absorption by plants increases when soil pH level in the rhizosphere rises. Havlin *et al.* (2005) and Polacco and Todd (2011) add that plants can store high concentrations of NO_3^-, but not NH_4^+, in their tissues. Havlin *et al.* (2005) explained that in protein synthesis, the process of NO_3^- reduction requires an energy source that utilises two NO_3^- reductase (NADH) molecules for every NO_3^- reduced. Therefore, NH_4^+ is the favoured N source to maintain the energy compared with NO_3^-, one less stage in the reduction process (Havlin *et al.*, 2005). In order to offset energy losses that occur during NO_3^- conversion, plants carry out this process at the same time as they photosynthesise. However, elevated concentrations of carbon dioxide (CO_2) in the atmosphere inhibit photorespiration and this either stops or reduces the absorption of nitrates (Foyer and Zhang, 2011, Bloom *et al.*, 2014). At the same time, soil pH must be managed by controlling the quantities of N fertilisers that are added to crops.

If N is present as NO_3^- or NH_3/NH_4^+ in the soil, conversion is not needed, as these forms are readily available to the plant. These sources are found in aquatic systems and N moves from the aquatic systems to the plant roots through mass flow or diffusion. In addition, N termed

'biological N' is present in components of living matter such as proteins and nucleic acids (Polacco and Todd, 2011).

2.5.1.3 Biological nitrogen fixation

Of all living organisms, only green plants and many micro-organisms contribute to the mineralisation and N fixation of raw materials (Epstein, 1971). As mentioned earlier, leguminous plants have a symbiotic relationship with rhizobial bacteria which fix N. In return, the plant supplies mineral elements and other organic compounds to the symbiotic bacteria. The amount of N fixed depends on the species of N-fixing bacteria, plant growth conditions, and the type of plant (Figure 2.1) (Espinoza *et al.*, 2005). For example, the bacterial species that are very effective with soybeans to fix N_2, are not effective with alfalfa. The process of N fixation occurs in the root nodules that form on the root system (Espinoza *et al.*, 2005). The process of biological N fixation is achieved by stimulating the nitrogenase enzyme, a process that is influenced by many soil and weather factors. The process of biological N fixation can cease or fall to minimal levels as a result of very low soil pH levels, or the presence of large quantities of available mineral N. This is because the symbiotic associations between the bacteria and the host plants do not function very well at these levels. The use of an inappropriate bacterial inoculum will reduce the formation of nodules, thereby impeding the process of biological N fixation. In fact, there are other organisms in the soil that are able to fix N_2 via non-symbiotic associations. However, the output of these organisms, with the exception of blue-green algae, is of minor importance (Espinoza *et al.*, 2005).

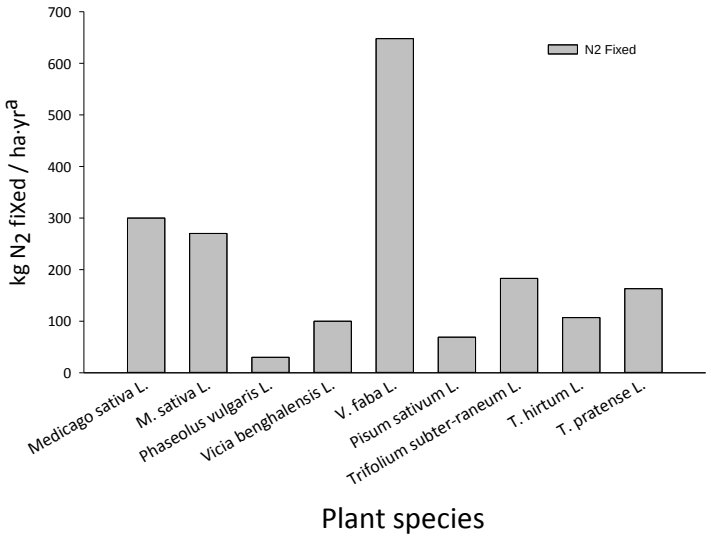

Figure 2.1 Efficiency of symbiotic N_2 fixation by some temperate legumes in the orchard, the data source is (Phillips, 1980). Methods were used to estimate N_2 fixation: [(1)] for "*Medicago sativa* L. used

soil and crop N balance for 10-yr continuous culture. [2] *M. sativa* L. and *T. pratense* L. used soil and crop N balance for 10-yr rotating culture (legumes rotated in alternate years with *Hordeum vulgare* L. or *Secale cereale* L.). [3] *Phaseolus vulgaris* L. used N difference between plots with and without *Rhizobium.* [4] *Vicia benghalensis* L. used ^{15}N A-value correction of total-N difference. [5] *V. faba* L. and *Pisum sativum* L. used C_2H_2 reduction. [6] *Trifolium subter-raneum* L. used ^{15}N A-value with *Bromus mollis* L. as a reference crop. [7] *T. hirtum* L. used ^{15}N A-value correction of total-N difference" (Phillips, 1980).

2.5.2 Phosphorus

Along with N and K, P is also a key nutrient for plants. Phosphorus forms part of the nucleus of the plant cell, and the plant needs it for the production of bio-energy and for cell division. It is also necessary for opening of the stomata (Lyle *et al.,* 2006), which may be due to the changes in the balance between cytokinins and abscisic acid, as result of the change in the level of P leaf (Radin, 1984). Because atmospheric returns of P are low compared with carbon and N, and biological P recycling is slow, agricultural fields are typically supplied almost entirely with artificial P (Walker and Syers, 1976, Ezawa *et al.*, 2002, Tipping *et al.*, 2014). Phosphorus is less mobile within both plants and soil compared to K; therefore, its deficiencies show up in young leaves. Also, unlike K, it is less easily leached from the soil (Lyle *et al.,* 2006). Fernandez and Rubio (2015) reported that a deficiency of P enhances a steady increase in root aerenchyma and root porosity of sunflower, maize, and soybean. Fernandez and Rubio (2015) stated that the formation of aerenchyma causes a decrease in or modifies root length/unit root biomass, which in turn leads to a decline in foraging by the roots (Fernandez and Rubio, 2015). It was also observed that the deficiency of P led to a decrease in the density of roots in some pasture species (Fernandez and Rubio, 2015). However, because P can easily be locked away in a form that is inaccessible to plants due to its ability to form complex molecules with other nutrients in the soil, Lyle *et al.* (2006) believe that P deficiency is related more to soil pH rather than to deficiency in the soil itself. We can conclude from this that the pH of soil plays a pivotal role in facilitating or fixing a range of nutrients, just as it does in the biological life of soil. As has been stated previously, soil pH is directly affected by the type of agricultural practices used, particularly conventional practices. Therefore, for soil to be a suitable medium for nutrients, pH must be maintained at an appropriate level. Hence finding suitable horticultural applications that will improve the soil pH and enhance productivity and plant growth is important.

Walker and Syers (1976) and Gyaneshwar *et al.* (2002) made two findings: firstly, that biological N fixation ceases under normal circumstances when available inorganic P had vanished, and secondly, that in the absence of inorganic P, non-nitrogen fixing organisms, either plants or microbes, compete with each other for the N and P mineralised from organic matter in the soil; as a result available inorganic P disappears or becomes limiting. However, these two issues can be solved if inorganic P is made available. This suggests that industrial input cannot be completely dispensed with. However, it is still feasible to minimise the negative effects of synthetic fertilisers by integrating specific rates of recommended applications with organic, biological alternatives.

It is well known that bacteria play a significant role in dissolving P in the soil for uptake by plants. However, studies by Banik and Dey (1982), Kucey (1983) and Turan *et al.* (2006) found that since most of these bacteria are able to solubilise calcium phosphates and few can solubilise iron phosphates and aluminium phosphates, they would be more effective in calcareous soils than in alfisols where phosphates are complexed with Fe and aluminium (Al). Further research is therefore needed to identify microbes that can solubilise these iron and aluminium phosphates and to mobilize phosphate reserves in the soil, so that more P is available to plants.

Fixed P in the soil requires several factors to become optimised, including sufficient moisture within the soil, appropriate soil pH, and biological activity in the soil. Gyaneshwar *et al.* (2002) reported that mycorrhizal fungi and P-solubilising bacteria produce organic acids such as acetate, lactate, glycolate, tartarate, oxalate, succinate, gluconate, citrate and ketogluconate which, in turn, affect the ability of phosphate to be solubilised. Most bio-dissolution of phosphate occurs for phosphate that has formed a complex compound with calcium (Ca), while small amounts of phosphate solubilise from P when the latter is combined with Fe and Al. Gyaneshwar *et al.* (2002) also suggest that mycorrhizal fungi may not be able to form strong colonies on plant roots in situations where high concentrations of P are present in both plants and soil. They also discovered that, in some cases, there was a reduction in growth of plants which had been inoculated with mycorrhizal fungi in the presence of high levels of available phosphorous. This may be attributed to the direct uptake route that plants use which may be prevented by AMF colonisation or there are few benefits to outweigh the impact of the C-sink. Both of these findings must be taken into account when trying to improve soil fertility to maintain biological sustainability.

2.5.2.1 The Phosphorus Cycle

Phosphorus can be classified into four forms or sources. These are inorganic P (which is artificially added yet becomes rapidly unavailable due to formation of complex molecules with other nutrients), organic P, adsorbed P and rock P in its initial form. The solubilising of P depends on the types of N sources in the surrounding environment. For example, it has been observed that a greater increase in solubility occurs in the presence of ammonium salts than in environments containing nitrates. This may be attributed to the launch of protons to compensate for the absorption of ammonium, thereby reducing extracellular pH (Roos and Luckner, 1984).

Organic-P is converted by soil microbes and fungi during the mineralisation process to phosphoric acid ($H_2PO_4^{-2}$) or hydrogen phosphate (HPO_4^{-2}) (Hyland *et al.*, 2005). Many plants have demonstrated the benefit of forming symbiotic associations with micro-organisms including bacteria and fungi under conditions of P-deficiency (Gyaneshwar *et al.*, 2002). Under these conditions, the micro-organisms can increase the absorption of available P, or work to solubilise insoluble mineral P complexes, especially calcium phosphate (Ca-P) complexes. The ability of micro-organisms to dissolve Ca-P complexes can be attributed to changes in soil pH through the release of organic acids or protons in the surrounding areas (Gyaneshwar *et al.*, 2002, Wu *et al.*, 2006). Gyaneshwar *et al.* (2002) reported that the mineral phosphate can either be directly dissolved by the organic acids secreted as a result of PO_4^{2-} anion exchange, or can chelate Al and Fe ions associated with phosphate.

2.5.3 Potassium

Potassium (K) is a mobile nutrient within plants and is required to perform many physiological processes such as water homeostasis, photosynthesis, protein synthesis, and subsequent conversion into oils, carbohydrates, and other products (Marschner, 1995, Havlin *et al.*, 2005, Marschner and Marschner, 2012). Additionally, K has primary responsibility in most plant species for turgor changes in the guard cells, thus impacts on stomata behaviour (Marschner, 1995, Marschner and Marschner, 2012). Potassium plays a role in cell permeability, and indirectly increases plant resistance to disease by influencing certain physiological processes of the plant as well as the biosynthesis of metabolites (Marschner, 1995, Lyle *et al.*, 2006, Marschner and Marschner, 2012, Srivastava, 2012).

While there is abundant K in the soil, it is often unavailable to plants because it most often forms complex compounds with other nutrients in the soil, as does P. Furthermore, supplies

of available K can be easily leached. Thus, deficiencies and availability can occur together, especially in light and sandy soils (Srivastava, 2012). Marschner and Marschner (2012) illustrate that K-deficiency leads to growth retardation and K re-translocation occurs from mature leaves and stems and under severely deficient conditions these organs become chlorotic and necrotic. Also, plants that have received insufficient amounts of K are often more likely to be damaged by frost. This damage caused by K-deficiency is relevant to the deficiency of water at the cellular level (Marschner and Marschner, 2012). Since K moves easily in the plant, deficiency symptoms appear on older leaves first as it is translocated to growing regions.

Kumar and Sharma (2013) state that symptoms of K-deficiency on sunflower plants (*Helianthus annuus* L.) are likely to occur when grown in soils formed from parent material with a low content of K, light textured soils that allow K to leach, low organic matter soils, acid soils (soil pH lower than 6) or high bicarbonate concentration in irrigation water and high rates of Mg:K, Na:K and Ca:K. The permeability of such soils should be improved with organic additives that reduce leaching of these minerals, or act as a sink for them. Moreover, it is necessary to also find a sound ground management plan to facilitate the release of these minerals which are locked up with other elements within the soil.

2.5.4 Calcium and Magnesium

Calcium

Calcium (Ca^{+2}), an immobile element in plants, is an important regulator of plant growth and development, being involved in many vital processes within the plant (Hepler, 2005). For example, it is essential for protein formation and metabolism of N by enhancing NO_3^- absorption; it also plays a key role in the translocation of nutrients and carbohydrates (Havlin *et al.*, 2014). Inadequate uptake of Ca^{+2} depletes the plant's store of carbohydrates in leaves, roots and stems. This decrease in root carbohydrate content, in particular, impairs root functions such as nutrient and water uptake (Havlin *et al.*, 2014). However, symptoms of Ca^{+2}-deficiency are limited under field conditions. Havlin *et al.* (2014) state that Ca^{+2} is important to cell elongation and division. Hepler (2005) reports that Ca^{+2} deficiency can result in a number of defects: poor root development, leaf curling, leaf necrosis, blossom end rot, fruit cracking, bitter pits, short storage-life of fruit, and water soaking. There are underlying causes of these symptoms: Ca^{+2} plays a major role in cross-linking acidic pectin residues, and a low concentration of Ca^{+2} increases the permeability of the plasma membrane

(Hepler, 2005), resulting in loss of cell contents and failure of nutrient-uptake mechanisms (Havlin *et al.*, 2014).

Ferree and Warrington (2003) state that symptoms of Ca^{+2} deficiency in apple appear primarily on fruits, rather than leaves. However, they noted that leaf deficiency symptoms are rarely observed under orchard conditions. In reporting the symptoms of Ca^{+2} deficiency in the apple cultivar 'York Imperial' grown in nutrient solutions with different Ca^{+2} concentrations. (Ferree and Warrington, 2003) observed progressive development of leaf symptoms from upwards cupping of the youngest leaves, followed by veinal and interveinal chlorosis, and finally the formation of chlorotic spots and necrotic tissues on the leaf edges, while symptoms in fruit included abnormal skin bronzing, darkening of lenticels, and splitting of fruit during harvesting.

Earlier reports by Webster and Looney (1996) indicated an absence of documentation of Ca^{+2} deficiencies in cherry orchards. They did note, however, symptoms that were experimentally induced in young trees included a light brown colour on the leaves, which later changed to yellow, the appearance of dead leaf tissue followed by numerous holes in the leaves, poor growth of shoots, and an increased tendency of mature fruits to crack when exposed to rain. Since Ca^{+2} is a non-mobile element which is not redistributed among different plant parts, nutrient application immediately prior to harvesting would most likely be ineffective in preventing deficiency symptoms in the fruit. However, foliar application of Ca^{+2} prior to harvesting may reduce the incidence of fruit cracking due to contact with rain, as reported by Webster and Looney (1996) and Ekinci *et al.* (2016).

Magnesium

Havlin *et al.* (2014) state that magnesium (Mg^{+2}) is a primary component of chlorophyll, accounting for 15-20% of the total chlorophyll biomass. Thus, it is vital to photosynthesis. Mg^{+2} is also a structural component of the ribosome needed for maximum activity of the phosphorylating enzyme involved in carbohydrate metabolism.

Unlike Ca^{+2}, Mg^{+2} is a mobile element within plants, moving from older to younger leaves. This explains why the symptoms of Mg^{+2} deficiency often appear on the lower leaves. Mg^{+2} deficiency, in most plants, causes brown interveinal leaf chlorosis and leaf yellowing. In severe cases, leaf tissues become uniformly chlorotic, then necrotic (Ferree and Warrington, 2003, Havlin *et al.*, 2014). These symptoms were demonstrated by Christin *et al.* (2009) using a hydroponic system. They noted that leaf numbers, plant height and root length all

decreased significantly in sunflower plants deficient in Mg^{+2}. By the end of the experimental period, the plants had stunted growth and brown chlorotic and necrotic leaves. Ferree and Warrington (2003) found that, in apple trees, such a deficiency is more apparent in leaf tissue (e.g. leaf curling, brown interveinal necrotic blotches, yellowing, premature defoliation and reduction in canopy photosynthesis). Ferree and Warrington (2003) established that the severity and expression of symptoms vary between cultivars. For example, 'Delicious' apple is less susceptible to Mg^{+2} deficiencies than 'McIntosh' apple. Troyanos *et al.* (2000) observed that the sensitivity of cherry trees to Mg^{+2} deficiency can differ, depending on cultivar. They also demonstrated that the response of cherry trees to Mg^{+2} deficiency can depend on root biomass and the age of the tree. For example, Mg^{+2} deficiency in young trees of *Prunus avium* 'F 12/1' results in a reduction in shoot growth and abscision of leaves, resulting in a greater risk of transplanted stock failing to thrive, and a consequent reduction in total fruit yield (Troyanos *et al.*, 2000).

Absorption: According to Chapin (1980) and Havlin *et al.* (2014), Ca and Mg cations are highly mobile in the soil, easily moving to the root surface through diffusion and mass flow. When the supply of these cations exceeds the roots' capacity to absorb them, the excess accumulates around the roots. The rate at which Ca^{+2} and Mg^{+2} is absorbed is affected by the rate of transpiration in the plant, and when the concentration of these minerals in the soil solution is extremely low, growth can be affected (Chapin, 1980). This can happen in unlimed acidic or highly leached soils (Havlin *et al.*, 2014). In addition, when the plant roots absorb N in the form of NH^{4+}, the charge imbalance created results in absorption of other cations at very low rates. Havlin *et al.* (2014) identified several factors that determine the availability of Ca^{+2} and Mg^{+2} to plants: soil pH, cation exchange capacity (CEC), %Ca or %Mg saturation, type of soil clay, and the ratio of Ca^{+2} or Mg^{+2} to other cations in solution. With regard to Ca^{+2} in particular, Havlin *et al.* (2014) found that soil conditions that decrease root growth (e.g. P-deficiency, Al^{+3} toxicity, diseases and pests) limit root access to Ca^{+2}, and thus induce a deficiency. Furthermore, when conditions causing plants to develop small root systemsalso result in impairment of Ca^{+2} uptake. For these reasons, identifying the most suitable horticultural practice for each particular crop or soil type is a vital first step in reducing the impediments to Ca absorption.

2.5.5 Micronutrients

As with macro-nutrients, micro-nutrients which include boron (B), chlorine (Cl), cobalt (Co), Cu, Fe, manganese (Mn), molybdenum (Mo), sodium (Na) and Zn, are also needed by plants, but in smaller quantities. Some fertilisers contain these nutrients but these are often applied in the form of foliar applications and in low concentrations. As with N, P and K, micro-nutrient absorption is affected by soil pH (Figure 2.2). A study by Marschner (1993) revealed soil pH is the determining factor in whether Zn in solution in the soil can be absorbed or not; the mobilisation and absorption of Zn decreases in acidic soils. Also, it was found that root secretions, or changes that occur on roots in the rhizosphere are important for the absorption of Zn from the soil. Marschner (1993) also discovered that high soil acidity resulting from excessive applications of N, or symbiotic N-fixation by leguminous crops affects the mobilization of Zn. These same conditions, as well as excretion of organic acids can also lead to P and Fe deficiencies. It was observed that mycorrhizal inoculation raises the levels of Zn and P in the dry shoot matter (Marschner, 1993). Thus, finding alternatives to synthetic N fertilisers or using fertilisers which release elements slowly may impede mycorrhizal colonisation and thus improve Zn and P levels in the soil.

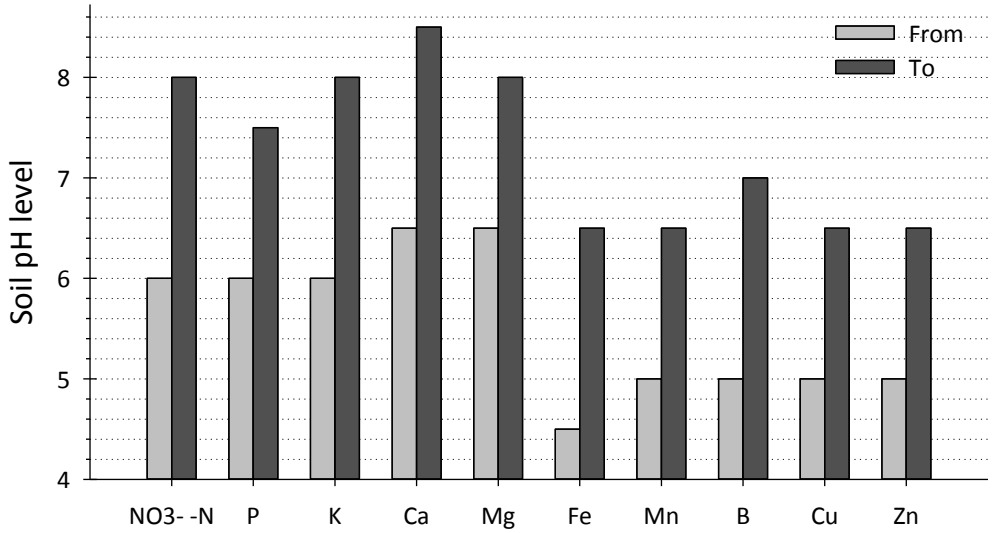

Figure 2.2 The relationship between soil pH and nutrient uptake by plant ('From' and 'To' indicate the range of availability). The figure is adapted from (Hyland *et al.*, 2005, Lyle *et al.*, 2006).

Plants also need small amounts of B; symptoms of B deficiency are evident under conditions of low soil moisture. In sunflower plant (*Helianthus annuus* L.), B-deficiencies can present as ceased growth and low productivity (Kumar and Sharma, 2013). As B is not mobilised from older to younger leaves, its deficiency appears clearly on younger leaves, while older leaves appear normal and healthy.

Plants require Co to fix N (Hewitt and Bond, 1966, Marschner and Marschner, 2012), especially in leguminous crops. In addition, Mn, Zn and Na are also needed for interactions between certain enzymes and growth hormones in plants (Kumar and Sharma, 2013), which greatly enhance drought tolerance. Copper, too, is important for respiration and it is essential for absorption of Fe by plants; Fe contributes indirectly to the process of photosynthesis, and small, pale leaves are a sign of deficiency of this element (Marschner and Marschner, 2012, Havlin *et al.*, 2014). Iron is present in the soil in large quantities, only needing an appropriate pH level in order to be available. Symptoms of Fe deficiency can be seen in plants, especially citrus, growing in most alkaline soils (Marschner and Marschner, 2012, Havlin *et al.*, 2014). Therefore, maintaining an appropriate pH of the soil is important to facilitate Fe uptake by plants.

2.5.6 The role of nutrients and carbon

Soil carbon (SC) is an essential component of agricultural soils, which is important for nutrient cycling in ecosystems, promoting biomass of the microorganisms. Soil C is also an important sink for greenhouse gases. Maintenance of the sustainability of agricultural lands and reduction of greenhouse gas emissions has prompted a push to find strategies that will manage soil C based on changing management practices or use of amendments, such as biochar additions (Atkinson *et al.*, 2010, Schulz and Glaser, 2012). Soil treatment with biochar has been claimed to be a suitable technique to mitigate climate change on a large scale through reducing emissions of greenhouse gases (Glaser *et al.*, 2002, Sohi *et al.*, 2010). Biochar can amend soil properties, resulting in positive changes in soil aeration, improving water relations and increasing the soil's ability to retain nutrients (Glaser *et al.*, 2002, Chan *et al.*, 2008, Atkinson *et al.*, 2010), and alter biological activity (Lehmann *et al.*, 2011).

Modifying the soil with C can hinder plant growth. From the investigation conducted by Novak *et al.* (2009), when the amount of added C increased (as biochar amendment), the release of some cations (e.g. Ca and Mg) decreased, especially during the first days following addition of the amendment (Figure 2.3a). The amount of the lost cations with leaching water decreased when the soil-C content increased (Figure 2.3a). By contrast, leaching water content of the cations (Ca, K and S) increased with increasing age of the added C (Figure 2.3b). This means that C amendments increased the release of these cations in the soil over time. The results of Novak *et al.* (2009) showed that soil-C amendments raise soil pH over time (Figure 2.3 c and d). Biochar can be used to reduce the toxicity of high levels of micro-

nutrients in soils. The concentration of Mn, Zn and Cu in leaching water decreased with increasing C additive and the length of time (Figure 2.3 c and d).

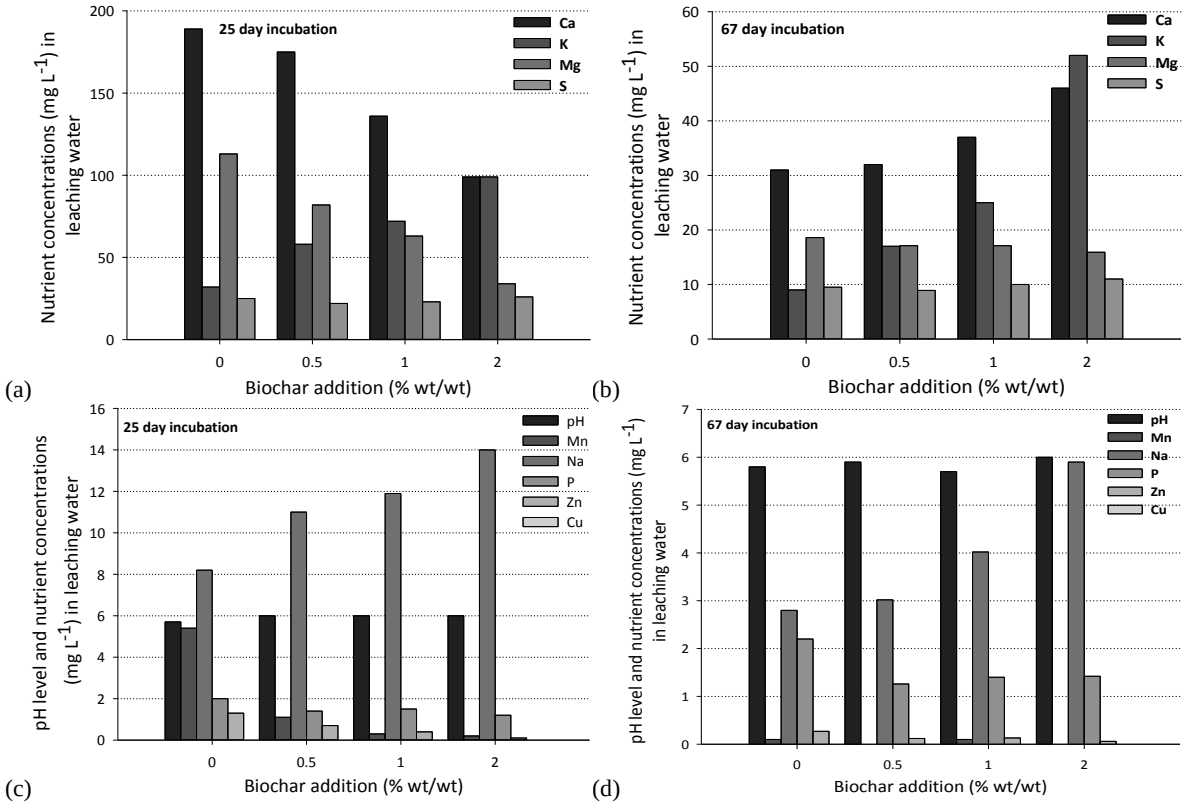

Figure 2.3 The impact of the change in soil carbon content on the amount of missing mineral nutrients with the leaching water, data were obtained from (Novak *et al.*, 2009). Soil information: Top field soil (0 – 15 cm deep) was collected after one week from fertilisation with 49 kg ha^{-1} N of 28-0-0 UAN (urea + NH_4NO_3). The field has a long history (30 years) of row crops. The soil type is loamy sand; particle size is 730, 250, and 20 g kg^{-1} of sand, silt, and clay respectively. Soil pH was 4.8. Notice: Cu value is zero.

Soils containing high amounts of organic carbon need appropriate management to provide phosphorus (P) for plant growth. It was observed that increasing concentrations of C in the soil reduced the available-P (Figures 2.4 c and d). Carbon amendments can also cause soil salinity; the increase in soil carbon content is accompanied by an increase in the release of sodium (Figures 2.4c and 2.4d).

It has been shown that biochar added to the soil has positive effects in the maintenance of soil nutrients (Glaser *et al.*, 2002, Lehmann *et al.*, 2003, Bélanger *et al.*, 2004, Major *et al.*, 2010), capacity of cation-exchange (Schulz and Glaser, 2012), the capacity of water retention (Glaser *et al.*, 2002), mycorrhizal and soil microbial activity (Warnock *et al.*, 2007, Schulz and Glaser, 2012), electric conductivity (Asai *et al.*, 2009), soil fertility (Lehmann *et al.*,

2003, Rondon *et al.*, 2007, Novotny *et al.*, 2009), and an expected influence on plant nutrition and growth (Lehmann *et al.*, 2003).

Novak *et al.* (2009) has demonstrated that biochar applications have the ability to improve the efficiency of macronutrient use mainly Ca (Figures 2.4 a and b), through acting as a sink of these nutrients, and/or by altering soil pH, but biochar has a low capability to improve the efficiency of micronutrient use (Figure 2.4 c and d).

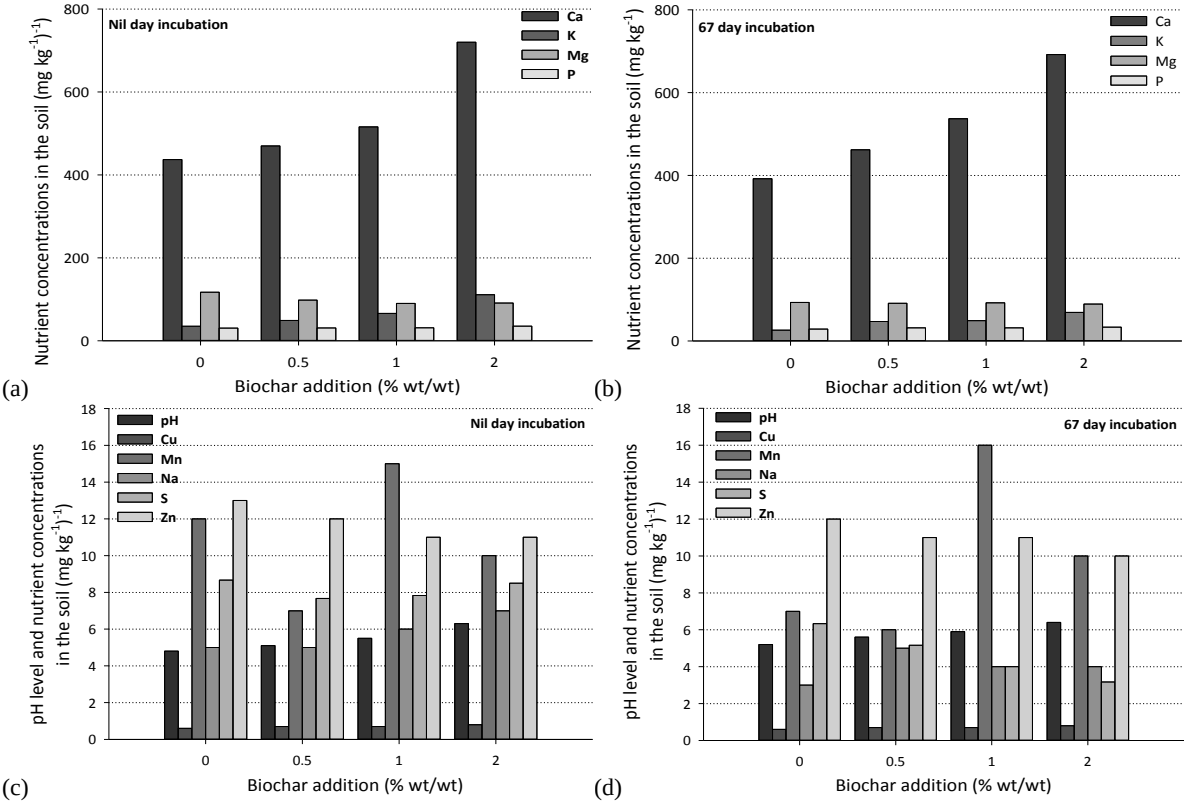

Figure 2.4 The impact of the change in soil carbon content on chemical characteristics of the soil, data were obtained from (Novak *et al.*, 2009). Soil information: Top field soil (0 – 15 cm deep) was collected after one week from fertilisation with 49 kg ha^{-1} N of 28-0-0 UAN (urea + NH_4NO_3). The field has a long history (30 years) of row crops. The soil type is loamy sand; a particle size is 730, 250, and 20 g kg^{-1} of sand, silt, and clay respectively. Soil pH was 4.8.

Carbon can be added to the soil naturally as a result of the decomposition of organic material by free-living soil microbes and mycorrhizal fungi (Moore *et al.*, 2015), or be added to the soil as an amendment (Schulz and Glaser, 2012). Microbes break down molecules of organic matter and as well as providing C to build microbial biomass, this mineralisation process supplies plants with essential nutrients for growth (Moore *et al.*, 2015). Also, the breakdown and mineralisation processes of organic matter in the soil result in the return of large amounts of C into the atmosphere as CO_2 (Fenn *et al.*, 2010).

Plant roots and mycorrhizae interact in different ways with the soil community and the results of these interactions in turn affect decomposition processes and microbial activity (Högberg *et al.*, 2007, Phillips *et al.*, 2012, Clemmensen *et al.*, 2013, Moore *et al.*, 2015). For instance, the roots and mycorrhizae exude C substrates in the soil that influence microbial communities and ecosystem functions (Bais *et al.*, 2006, Phillips, 2007, De Graaff *et al.*, 2010). Secretions of roots and microbial communities increase the decomposition of old organic materials (Kuzyakov, 2010) and mineralise nutrients that will be ready for plant uptake.

2.6 Climate

Climatic factors, particularly moisture and temperature, have a major effect on biological processes in both soil and plants. Adequate moisture levels allow microbial activity and biological processes to occur and water also enables plants to transfer nutrient elements to and from the soil. Piccolo *et al.* (1997) stated that soil moisture has the most important role in this transport. Cyclic wetting and drying affects the structural stability of the soil; it may also have an impact on the bulk density of the soil, and can lead to aggregation of small particles together, forming larger particles. Large aggregates formed from a pool of small aggregates may disintegrate again into smaller aggregates as a result of the fractures that arise during the expansion and contraction of the soil. These contractions and fissures that occur in the soil have consequences on the overall stability, reducing or increasing infiltration rates, often leading to erosion of the surface of the soil due to runoff (Piccolo *et al.*, 1997). Both animal and plant residues enhance soil structure and may reduce the risk of leaching as organic matter is considered a sink for nutrients (Welbaum *et al.*, 2004, Manlay *et al.*, 2007). Excessive precipitation causes leaching of nutrients from the soil, acid rain increases the soil's acidity, and water-logging raises the level of ground water, resulting in soil salinisation in many soils (Salama *et al.*, 1999, Dahlhaus *et al.*, 2000, Nickson *et al.*, 2005).

Chikowo *et al.* (2004) observed that high humidity during the rainy season, in sandy textured soils in Zimbabwe, enables more inorganic than organic N in the soil to be absorbed and mineralised. This increase in mineralisation and absorption may be the result of interaction between traditional compounds and the remnants of fertilisers in the soil which are released as a result of the increase in soil moisture. Working on beech forests, Meier and Leuschner (2014) found that the rate of soil fertility changed in locations that have summer rains, and concluded that the decrease in soil acidity and increased base saturation in forest soils resulted in an increase in the decomposition activity. This increase in the decomposition activity reduced N resorption and increased the ratios of N/P and N/K, which were lower

before the precipitation. Increased N/P and N/K ratios are very important to growing plants (Meier and Leuschner, 2014). These authors also observed that K and P content of leaves increased with the increase in soil moisture (due to precipitation) in forest soils, and the possible reason behind this is the high availability of NO_3^- resulting from a reduction of soil leaching. They concluded that soil moisture affects the formation of the mycorrhizal community, which in turn enhances the absorption of mineral nutrients.

Rainfall and temperature also have an impact on the level of organic carbon in the soil. Zhao *et al.* (2015) and Fortin *et al.* (2011) have observed that organic carbon content is related to temperature and the amount of precipitation. The change in temperature that occurs with higher precipitation causes a rise in organic carbon. This is due to increases both in decomposition of organic matter and microbial activity in the soil. Relatively low temperatures give the same results as using conventional applications, namely, an imbalance of the various organisms in the soil. Elkoca *et al.* (2007) found that excessive inoculation with PGPR could be more effective for production, particularly in soils located in relatively cold highland areas. This could be a good way to overcome limited activity of microbes in these regions. According to Elkoca *et al.* (2007), a number of experiments conducted to determine the effectiveness of bio-fertilisers as alternative sources of nutrients has shown that using bio-fertilisers to inoculate plant roots gives the best results in sub-tropical and warm climates compared to cold temperature conditions. This result indicates that the temperatures in the rhizosphere were appropriate for these microbes. It is known that organic residues in the soil require a considerable amount of energy to decompose. This thermal energy released from the decomposition process causes a temperature rise in the rhizosphere, providing a suitable environment for biological activity. Therefore, in temperate and cold climates, excessive inoculation can be successful if sufficient amounts of nutrients are added to avoid competition. Schimel and Bennett (2004) reported that plants are weak competitors for available soil N compared to soil microbes; hence microbes may deplete soils which have low available N, leaving insufficient N for plant growth.

Adequate moisture is not the only factor required for biological processes; suitable temperatures and commensal symbiotic life forms in the soil are also required. It is well known that a large part of the process of mineralisation and transformation of elements is performed by bacteria and fungi in the soil (Singleton and Sainsbury, 2006, Boukli *et al.*, 2007). Close and Beadle (2004) noticed that increased levels of soluble N are closely associated with spring temperatures. However, while adequate warmth is important for these

interactions, extremes of temperature can be harmful. High temperatures speed up water evaporation and can affect nutrient movement. Droughts caused by high temperatures impact negatively on biological activity in the soil (Quilchano and Marañón, 2002) and on fertility in general. Planting cover crops such as clover under trees can be a way of maintaining moderate temperatures by ameliorating the effect of low or high ambient temperatures (Drury *et al.*, 1999, Dabney *et al.*, 2001).

2.7 Agricultural systems

The increased need for food globally has led to the expansion and intensification of agricultural land use, which has resulted in a decline in productivity of soils (Foley *et al.*, 2005). Sustainable future use requires careful land management and interventions to prevent decline in productivity. To maintain optimal nutrition, there are different options for amendments and other land management practices.

2.7.1 Types of agricultural systems

Conventional agricultural systems rely on synthetic inputs to provide plants with necessary nutrients for growth, and application of herbicides and pesticides to control weeds and pathogens respectively (Reganold *et al.*, 1990, Glover *et al.*, 2000, Brandt and Mølgaard, 2001, Dabney *et al.*, 2001, Trewavas, 2001, De Ponti *et al.*, 2012). For biological and organic systems, synthetic inputs are minimal or not used at all in the case of "certified organic" status (Reganold *et al.*, 2001, De Ponti *et al.*, 2012). Rather, for nutrition, plant and animal residues and compost provide plants with important elements, cover crops (leguminous plants) are used to fix N from the atmosphere, natural sources such as rock dust are used to supply nutrients such as P, and biological inoculations may be used to enhance nutrient uptake and mineral recycling in the soil (Clark *et al.*, 1999a, Clark *et al.*, 1999b, De Ponti *et al.*, 2012). Pest and weed control is limited to cultural methods (e.g. resistant plant varieties and tillage to control weeds) and biological controls (Brandt and Mølgaard, 2001, Dabney *et al.*, 2001, Tilman *et al.*, 2002, Adesemoye *et al.*, 2008, Nishanth and Biswas, 2008, De Ponti *et al.*, 2012). Integrated systems combine the best of conventional management systems and organic systems for sustainable agricultural land management (Matson *et al.*, 1997, Glover *et al.*, 2000, Reganold *et al.*, 2001, Adesemoye *et al.*, 2008, De Ponti *et al.*, 2012).

There is increasing awareness of the role of organic matter (OM) in soil health and fertility. Organic matter content (e.g. compost, soil organic carbon, humic substances) differs between soils, depending on the prevailing climatic conditions, methods of soil management and soil

clay content (Novotny et al., 1999, Shepherd et al., 2001, Cotching, 2006). Rose *et al.* (2013) stated that monoculture cropping and the use of synthetic fertilisers also cause a decrease in organic matter, resulting in a decline in the level of humic substances which play an important role in maintaining key soil functions and plant productivity (Lal, 2004, Sparling *et al.*, 2006). This decline is often followed by negative effects on soil health and productivity. It also has been noted by Cotching (2006) that rotations of crops have led to a decline in organic matter in both Tasmania and New Zealand.

2.7.2 The consequences of the use of each agricultural system alone

While conventional agriculture relies on synthetic inputs which may not be sustainable, there is much evidence that a shift from conventional to biological agricultural systems can result in a significant reduction in crop yields (Oberson *et al.*, 1993, Reganold *et al.*, 2001). Several studies pointed out that after a long period of using conventional systems there is a risk to the sustainability of the productivity of agricultural soils and environmental consequences, including: an imbalance in the microbial biomass of the soil, increased soil erosion, declining soil fertility, reduced biodiversity, groundwater contamination and the impact on the constituents of the atmosphere such as nitrogen oxides and carbon dioxide (Matson *et al.*, 1997, Barrow, 2012).

Welbaum *et al.* (2004) note that following the development of chemical fertilisers they proceeded to replace organic farming systems; however, the downside of this was that these fertilisers affected micro-organisms whose importance was still unrecognised at that time. Following the initial introduction of chemical fertilisers to agriculture after World War 2 crops flourished, with increased yields and improved quality. However, this success was short-lived as continuous and intensive use of agricultural fields eventually depleted the soil's nutrients which were either absorbed by the crops or washed away by rain or irrigation. The result was a decline in soil quality. This prompted even heavier applications of synthetic fertilisers and so on, in a vicious cycle that, while producing the desired results of increased crop quality and quantity, fixed large amounts of mineral nutrients to the soil, which causes damage to the soil, environment and human health in the long term.

Conventional agricultural practices often cause an imbalance in the population of organisms in the soil (Franke-Snyder *et al.*, 2001, Araújo *et al.*, 2009, Bainard *et al.*, 2011). The effect may be competition among different species for available resources; conventional practices also involve high usage rates of elements such as N or P which may impede the growth of

some species, for example, fungal hyphae of mycorrhizal fungi (Egerton-Warburton and Allen, 2000, Egerton-Warburton *et al.*, 2001, Adesemoye *et al.*, 2008). In addition, pesticides may also interfere with the growth of fungal hyphae on the roots of host plants or impact on the establishment of bacterial colonies by inhibiting the growth of the roots of the host plant (Bethlenfalvay *et al.*, 1996, Rejon *et al.*, 1997). The use of fungicides in conventional practices can also kill beneficial fungi (Beyer-Ericson *et al.*, 1991).

Biological systems also have some disadvantages. For example, mechanical weeding, or tillage, that is carried out when herbicides are not used can wipe out mycorrhizal colonies (Douds *et al.*, 1995, Gosling *et al.*, 2006, Rasmann *et al.*, 2009). However, in waterlogged soil weed control through tillage enhances soil quality by improving aeration, thus increasing microbial activity. However, ploughing during the growing season and/or during the period of mycorrhizal fungi activity may produce undesirable results, such as severing of the fungi's hyphae. It can be concluded from this that colonisation of beneficial fungi is best maintained by careful management of the timing of agricultural processes, such as tillage. Fontaine *et al.* (2003) reported that the nutrient content of organic matter used as fertiliser is dependent on the materials used. For instance, the result of the decomposition of straw residues often produces limited N (Fontaine *et al.*, 2003).

A considerable body of research has indicated that non-traditional systems, such as organic regimes, cannot promote plant growth by providing the required nutrients with the same speed as conventional systems. This is because organic fertilisers may provide sufficient N for the soil, and increase soil pH as a result of C-mineralisation, the production of OH^- ions through ligand exchange and the introduction of base cations such as K^+, Ca^{2+} and Mg^{2+} (Hargreaves *et al.*, 2008). An increase in soil pH may enable some of the nutrients to be absorbed or exchanged by the plants. However, organic fertilisers will not be able to provide enough of all nutrients, or facilitate increased availability of nutrients that are not present in sufficient quantities in the soil by improving soil pH. Therefore, dispensing with conventional systems is difficult when intensive production with high yields is needed in order to provide food in large quantities. On the other hand, when there is limited agriculture that does not strain the resources of agricultural soils, and when appropriate agricultural cycles include land fallow, it may be possible to dispense with such systems. The search is continuing to confirm the feasibility of replacing conventional practices, or merging them with organic and bio-systems, in order to minimise the environmental risks posed by the use of artificial or manufactured fertilisers, and to protect the soil from erosion and degradation. However, the

shift from traditional agriculture to biological agriculture leads to a significant reduction yield in agricultural crops (Oberson *et al.*, 1993), because plant nutrition management differs between the two types of agricultural systems. In traditional agriculture, the goal of fertilisation is the direct feeding of the plant through the supply of artificial sources of nutrients, often applied in large quantities. On farms managed using biological farming principles, the emphasis is on addition of organic matter and bio-inoculants to aid in mineralisation and feed the soil. Finding alternative ways of meeting the nutrient needs of plants is likely to improve both crop quality and soil fertility. Despite the fact that alternative methods may mean an increase in production costs in the short term (e.g. labourers are required to remove weeds instead of using herbicides), the long-term benefits are likely to be reduced labour costs and improved sustainability (Reganold *et al.*, 1990).

2.8 **Comparison of nutritional requirements between herbaceous annuals and woody deciduous perennials**

2.8.1 Nutrient concentrations

Perennial horticultural crops have vastly different growing strategies, and therefore nutritional needs, compared to annual crops. Woody perennials such as temperate fruit trees are deciduous and therefore nutritional needs reduce over colder months. Woody tissues can also act as a store for nutrients and mobile elements can be accessed as needed. Perennial crops are subjected to fewer disturbances (e.g. tillage) and therefore soil microbes are likely to be less disturbed. Annual crops are subject to more intensive management over shorter periods of time. They may be grown in a rotation with other crops and therefore nutritional needs may be synchronised with those of the crop planted before or after (e.g. leguminous crops may be grown prior to crops with particularly high N demands).

Plant growth in most ecosystems responds positively to an increase in nutrient availability (Gilliam, 2014), but it is difficult to determine nutrient requirements in most of the various ecosystems, because nutrient requirements are dependent on plant growth and the time scale for the full growth rate (Güsewell, 2004).

Gilliam (2014) reported that concentrations of some foliar nutrients in leaves of forest herbs are higher than leaves of woody plants for the same site. This variation in nutrient concentration is confirmed by several studies (Siccama *et al.*, 1970, Gilliam, 2014). According to data presented by Siccama *et al.* (1970) and Gilliam (2014), herbaceous crops require less N, K and Ca compared with woody crops, but more P and Mg (Figure 2.5).

Nutrient requirements also differ in herbaceous crops depending on crop type, life cycle and season (Siccama *et al.*, 1970, Gilliam, 2014).

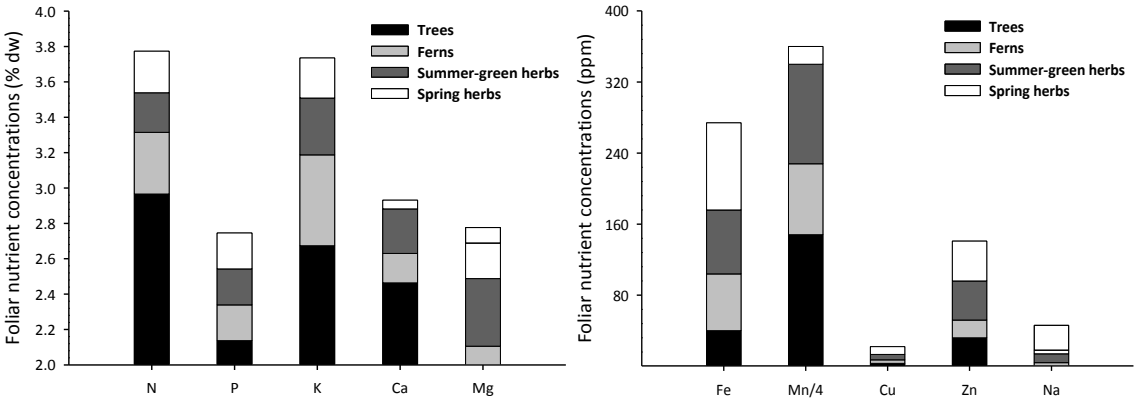

Figure 2.5 The average concentration of nutrients in the leaves of forest trees, cryptophytes (ferns), **"***summer-green herbs (completing photosynthesis during the summer growing season), and spring herbs (completing photosynthesis before canopy development) in the northern hardwood forest at Hubbard Brook, New Hampshire***"**. The leaves of plants were collected in the middle of the growing season for the respective phenological groups. **"**Data from Siccama *et al.* (1970). Nitrogen data for spring ephemerals is from a single species. *Erythronium americanum* (Muller 1978)**"** figure is adapted from Gilliam (2014).

In comparing nutrient levels reported in sunflower (Madejón *et al.*, 2003) and apple leaves (Peck *et al.*, 2006), it appears that concentration of K and Ca can be twice as high in apple, which is in agreement with the data presented by Siccama *et al.* (1970) and Gilliam (2014) for forest trees. Madejón *et al.* (2003) also observed differences in nutrient levels at different growth stages in sunflower, with seedlings having three times greater N levels than mature plants, but half the amount of Ca.

2.8.2 Nutrient uptake

Nutrient uptake also differs between herbaceous and woody perennial plants. Patterns of mineral nutrient uptake by herbs of deciduous forests widely rely on phylogenetic relations of individual species and phenological properties (Gilliam, 2014). Numerous deciduous forest herbs are host plants of mycorrhizae (Gilliam, 2014), which is useful for both the absorption of nutrients and water (Augé, 2001, Augé, 2004, Kumar *et al.*, 2010, Augé *et al.*, 2015). Species which are not hosts of mycorrhizae can be characterised by the length of their root hairs which can provide compensation (Gilliam, 2014).

Uptake and accumulation of nutrients in annuals has been reported to vary from species to species and from season to season (Gilliam, 2014). For instance, despite the existence of

mycorrhizal colonisation, nutrient uptake and accumulation in *Erythronium americanum* and *Allium tricoccum* (both spring ephemerals) are slow early in the season due to chilled soil in winter; but increase significantly later in the season (Gilliam, 2014).

Leguminous cover crops improve the N nutrition of fruit trees through biological N fixation (Lehmann *et al.*, 1999). The use of cover crops in orchard floor management can impact on nutrient status of tree crops, as the absorption of nutrients by cover crops is very high, and hence competition occurs between cover crops and fruit trees, especially with young trees (Lehmann *et al.*, 1999). Sánchez *et al.* (2007) reported lower leaf levels of N in young peach and apple trees grown with mowed cover crops compared to other soil management systems. Leaf content of N increased in coffee trees in an orchard managed with cover crops, while the leaf content of P, Ca, K and Mg decreased as a result of competition between the cover crops and trees (Lehmann *et al.*, 1999). Also, Marsh *et al.* (1996) observed that leaf content of K decreased in apple trees in orchards managed with cover crops. It is feasible that the big difference in patterns of nutrient absorption in deciduous forest herbs and cover crops (legumes and non-legumes) can be used to aid in the selection of appropriate agricultural practices and methods to assist in nutrient management.

2.9 Conclusion

The goal of horticultural practices is working to raise the soil's ability to carry out its functions in order to maintain productivity, preserve the environment and promote plant, animal and human health. In addition to other factors such as climatic conditions, horticultural practices can lead to enhancement or deterioration of soil health. The goal of the search for suitable soil management practices is to ensure the overall stability of the soil, including stability of biological activity as well as physical and chemical soil properties with the ultimate aim of improving agricultural productivity in the long term while ensuring long-term sustainability.

As discussed by Cotching (2006), organic matter is vital in the maintenance of healthy productive soils, and this review has highlighted the benefits of organic supplements and bio-fertilisers in maximising crop productivity. However, while there is an increasing interest in alternative nutritional management strategies which better promote soil health and previous research has highlighted the benefits of alternative nutrient management strategies on soil biota, including mycorrhizal fungi which are important symbionts of many crops, there is a gap in studies which objectively compare many of these alternatives. Hence this study aims

to clarify the difference between conventional fertilisers and alternative nutrient regimes high in organic matter on mycorrhiza and plant growth in two systems, sunflower and perennial deciduous tree crops.

"Chapter 3" Effect of humic based soil conditioner, effective microbes and fertiliser on growth and flowering of sunflower (*Helianthus annuus*. L. 'Dwarf Sunsation')

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3.1 Abstract

Biological farming methods are becoming more widespread as many farmers move towards the application of composts, bio-fertilisers and other organic additives. To assess the impact of coal-based humus and effective microbes on plant growth, two trials were undertaken on sunflower (*Helianthus annuus L.*, 'Dwarf Sunsation'). Two-week-old seedlings were planted into 16 cm diameter pots containing a basic potting mix plus Ferbon®, a lignite-based soil conditioner, at 0, 0.3 and 0.6 g per pot (equivalent to 0, 150 and 300 kg ha^{-1} respectively) in Trial 1, and 0 or 0.9 g Ferbon® (equivalent to 450 kg ha^{-1}) in Trial 2. After planting, pots were placed on glasshouse benches arranged in a randomised block design with six replicates per treatment for both experiments. Activated effective microbes (EM-1, Vital Resource Management Pty Ltd) were applied as a soil drench at 15 L ha^{-1} to half the pots after planting in Trial 1, and 0, 15 or 30 L ha^{-1} to pots in Trial 2. Pots were fertilised at weekly intervals with Hoagland's solution at 0, 50 and 100% concentration in Trial 1, or 0 and 100% in Trial 2. The label rate of 15 L ha^{-1} EM increased the number of nodes and stem height. Ferbon® had no effect on node numbers, but did increase stem height (Trial 1). The full rate of fertiliser resulted in increased stem height during the first 6 weeks of growth, but by week 9, the 50% fertiliser rate produced the same results as the full rate. Plants treated with EM displayed reduced leaf chlorophyll content compared with untreated plants. This reduction may be a function of increased biomass, evidenced by increase in plant height and stem diameter, deploying nitrogen. Ferbon® had no effect on chlorophyll content. Applications of both EM and Ferbon resulted in earlier flowering. These results demonstrate that with the availability of adequate accessible nutrients, both effective microbes and lignite-based humates such as Ferbon have the potential to increase plant productivity.

Keywords: Inoculation, Hoagland's solution, organic additives, chlorophyll content

3.2 Introduction

Biological farming methods are becoming more widespread as many farmers move towards the application of compost, bio-fertilisers, bio-stimulants and other organic additives. While there is no doubt that the introduction of synthetic inorganic fertilisers has significantly improved crop yields and allowed the development of large-scale intensive farming, there is increasing evidence that the continued reliance on synthetic fertilisers has longer term negative environmental consequences, including reduction in soil biological activities, soil erosion and contamination of groundwater (Barrow, 2012).

Organic substrates used as fertilisers are derived from plant and animal sources, and may have rock dust containing mineral nutrients added. Many nutrients in organic fertilisers need to be converted into inorganic forms by soil microbes before they become available for uptake by plants, hence are typically released slowly over time. Inorganic fertilisers are manufactured from minerals or synthetic chemicals. While they have the advantage that nutrients are in a concentrated form readily available to plants, they are often rapidly lost from the soil, and are more likely to cause more environmental problems than organic materials (Reganold *et al.*, 1993, Welbaum *et al.*, 2004).

Bio-stimulants are defined by Russo and Berlyn (1991) as non-fertiliser products which have a beneficial effect on plant growth. These authors also report that bio-stimulant blends have the potential to significantly reduce fertiliser use while still maintaining yield. Khaliq *et al.* (2006) state that inoculation of soil with effective micro-organisms (EM) along with organic or inorganic materials is an effective technique for stimulating supply and release of nutrients from these materials. EM is a mixed culture of active anaerobic and aerobic microbes; the most prominent organisms are photosynthetic bacteria, lactic acid bacteria and yeast.

There is considerable evidence that a transition from traditional to biological agricultural practices can lead to a significant decrease in crop yields (Oberson *et al.*, 1993, Reganold *et al.*, 2001). However, several studies have demonstrated that organic systems are able to achieve high fertility and high yields in the longer term (Granstedt and Kjellenberg, 1997, Glover *et al.*, 2000, Reganold *et al.*, 2001). As traditional and organic systems both have benefits, the challenge is to integrate these systems in such a way as to maximise the beneficial aspects of each system, while limiting their respective detrimental effects. The objective of this study was to examine the impact of chemical fertilisers, coal-based humate and effective microbes on growth of container grown plants.

3.3 Materials and Methods

Two trials were carried out under natural light conditions in glasshouses located in Hobart, Tasmania, from July to October 2013 (Trial 1), and December 2013 to March 2014 (Trial 2).

Sunflower (*Helianthus annus L.*, 'Dwarf Sunsation') seeds were germinated in trays in a basic potting mix (composted pine bark 80% by volume, coarse sand 20%, lime 3 kg/m^3 and dolomite 3 kg/m^3 for 2 weeks, and then transferred to 16 cm pots filled with 1.3 L of the same potting mix. Pots were placed on glasshouse benches and watered daily.

Trial design was a randomised complete block with six replicates per treatment. In both trials, treatments consisted of combinations of: (1) activated effective microbes (EM-1, sourced from Vital Resource Management Pty Ltd). EM-1 is a new product developed and produced within Australia to achieve microbial balance in soil and water. EM is a mixed culture of fermentative including active anaerobic and aerobic microbes, and lactic acid bacteria, photosynthetic bacteria and yeast are the most prominent organisms; (2) a lignite-based soil conditioner (Ferbon®, sourced from Interstate Energy Group, Bacchus Marsh, Victoria); and (3) liquid inorganic fertiliser (LIF) in the form of Hoagland's solution (Hoagland and Arnon, 1950).

Trial 1: Treatment design was a factorial arrangement of two EM rates (0 or 15 L/ha), three Ferbon applications (0, 0.3 or 0.6 g/pot, equivalent to 0, 150 or 300 kg/ha) and three LIF rates (0, 50% or 100% Hoagland's solution).

Trial 2: Treatment design was a factorial arrangement of three EM rates (0, 15 or 30 L/ha), two Ferbon applications (0 or 0.9 g/pot, equivalent to 0 or 450 kg/ha) and two LIF rates (0 or 100% Hoagland's solution).

3.3.1 Treatment application

The selected Ferbon amendments were spread evenly over the surface of the potting mix. Hoagland nutrient solution was applied weekly as a soil drench after watering; a total of 400 mL liquid was applied to each pot (400 mL Hoagland's for the full rate, 200 mL water plus 200 mL Hoagland's for the 50% rate, and 400 mL water for the control treatment).

The activated effective microbe solution (EM) was prepared according to the manufacturer's directions. The inoculation of EM was prepared by adding 30 ml of activated EM to 970 mL water, and 60 ml to 940 mL water for Trial 1 and Trial 2 respectively.

Solutions were applied as a soil drench after transplanting the seedlings, and again 6 weeks later.

3.3.2 Assessments

Leaf chlorophyll content was estimated with a Minolta SPAD-502 meter in weeks 5, 7 and 9 in both trials. Plant height and number of nodes were recorded at the same time. Stem diameter 2 cm above soil level, diameter of the primary flower heads and number of axillary flowers were measured in week 11 (Trial 1) and week 12 (Trial 2). Flowering dates were recorded daily from the beginning of flowering until the last plant flowered.

3.3.3 Data analysis

Data were subjected to analysis of variance using GenStat 14.2 (VSN International Ltd.), and presented as mean values for each treatment combination. Significance was calculated at $P = 0.05$ and least significant difference (LSD) used for comparison of treatment means in the tables and figures. Graphs were drawn using SigmaPlot 12.5 (Systat Software Inc.).

3.4 Results

Leaf chlorophyll content was reduced in EM inoculated plants in Trial 1 (Table 3.1), but remained unaffected in Trial 2 (Table 3.2). Addition of Ferbon had no effect on chlorophyll content in either trial. In Trial 1, 50% LIF increased leaf chlorophyll content compared with the control and 100% LIF treatments. However, in Trial 2, LIF had no effect on leaf chlorophyll content compared with the control (Table 3.2). A significant relationship was noted on leaf chlorophyll content between EM and LIF in Trial 1 (Figure 3.1a), and between EM and Ferbon in Trial 2 (Figure 3.1b). There was also a significant interaction between EM, Ferbon and LIF on leaf chlorophyll content in Trial 2 (Figure 3.2a).

Plant height was significantly increased by the addition of 15 L/ha EM in both trials (Table 3.1 & 3.2), but in Trial 2 the double rate of 30 L/ha had no effect (Table 3.2). Plant height was unaffected by Ferbon in either trial (Table 3.1 & 3.2), but the application of 100% LIF increased plant height in both trials (Table 3.1 & 3.2). There was a significant interaction between EM and LIF and plant height in Trial 2; EM combined with 100% LIF produced the tallest plants, but the higher EM rate reduced plant height compared with the lower rate; height was also significantly reduced in EM treatments without LIF compared with those with LIF (Figure 3.2b).

Stem diameter was increased by EM inoculation in Trial 1 (Table 3.1), but decreased in Trial 2 (Table 3.2). Ferbon had no effect on stem diameter in either trial. In both trials, stem diameter increased with increasing rate of LIF. There was a significant interaction between EM and LIF and stem diameter in Trial 1 (Figure 3.3a).

Table 3.1 Effects of activated effective microbes, Ferbon and liquid inorganic fertiliser (Hoagland's solution) in Trial 1 on leaf chlorophyll content, plant height, stem diameter, node number, flowering time, flower number and flower head diameter of sunflower (*Helianthus annus L.* 'Dwarf Sunsation').

	Leaf chlorophyll content (SPAD unit)	Plant height (cm)	Stem Diameter (mm)	Node #	Flowering time (days)	Flower #	Flower Diameter (mm)
(i) Effective microbes							
nil	33.4 a	14.9 b	8.4 b	11.1 b	71.4 a	1.1 b	43.1
15 L/ha	31.0 b	17.9 a	9.1 a	13.1 a	67.9 b	1.3 a	41.0
(ii) Ferbon							
nil	32.0	15.1 b	8.8	11.8	72.2 a	1.3	40.6 b
150 kg/ha	32.9	16.1 ab	9.1	12.2	70.3 a	1.2	44.8 a
300 kg/ha	31.6	18.0 a	8.4	12.4	66.4 b	1.1	40.7 b
(iii) Liquid inorganic fertiliser concentration							
nil	31.3 b	13.8 b	7.1 c	10.2 b	71.9	1.0 b	35.3 b
50%	35.5 a	17.0 ab	9.1 b	13.6 a	69.5	1.2 b	45.8 a
100%	29.7 b	18.3 a	10.1 a	12.6 a	67.5	1.5 a	45.0 a

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 3.2 Effects of activated effective microbes, Ferbon and liquid inorganic fertiliser (Hoagland's solution) in Trial 2 on leaf chlorophyll content, plant height, stem diameter, node number, flowering time, flower number and flower head diameter of sunflower (*Helianthus annus* L. 'Dwarf Sunsation').

	Leaf chlorophyll content (SPAD unit)	Plant height (cm)	Stem Diameter (mm)	Node #	Flowering time (days)	Flower #	Flower Diameter (mm)
(i) Effective microbes							
nil	30.4	19.6 b	8.8 a	14.1	69.7 a	1.8	62.4
15 L/ha	30.1	23.9 a	7.8 b	14.4	65.9 b	1.4	61.3
30 L/ha	30.9	19.8 b	8.1 b	13.0	67.7 ab	1.9	58.0
(ii) Ferbon rate							
nil	31.2	20.9	8.3	13.5	67.1	2.0 a	58.8 b
450 kg/ha	29.8	21.4	8.2	14.2	68.4	1.4 b	62.4 a
(iii) Liquid inorganic fertiliser concentration							
nil	30.1	10.2 b	5.5 b	9.9 b	72.7 a	1.0 b	35.6 b
100%	30.9	32.1 a	11.0 a	17.7 a	62.8 b	2.4 a	85.6 a

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

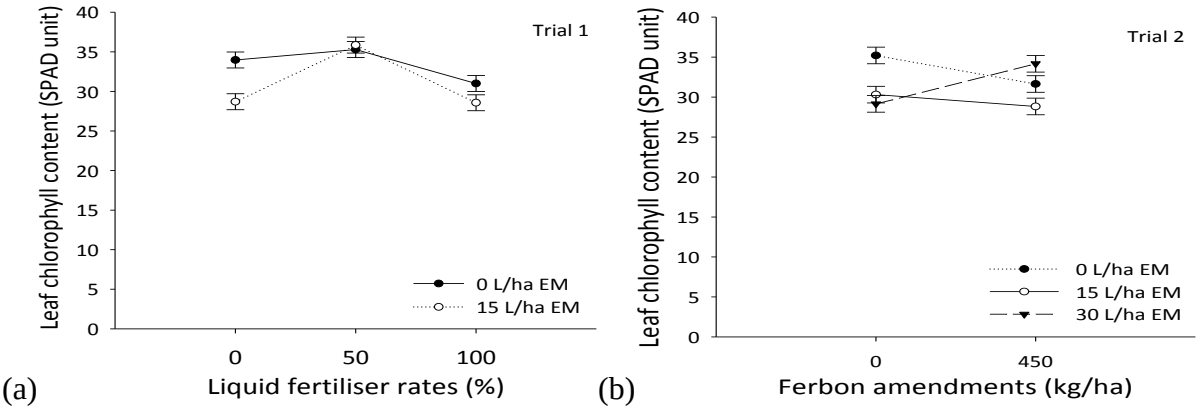


Figure 3.1 Effects of the interaction between (a) effective microbes (EM) and liquid inorganic fertiliser (Trial 1), and (b) EM and Ferbon (Trial 2) on leaf chlorophyll content.

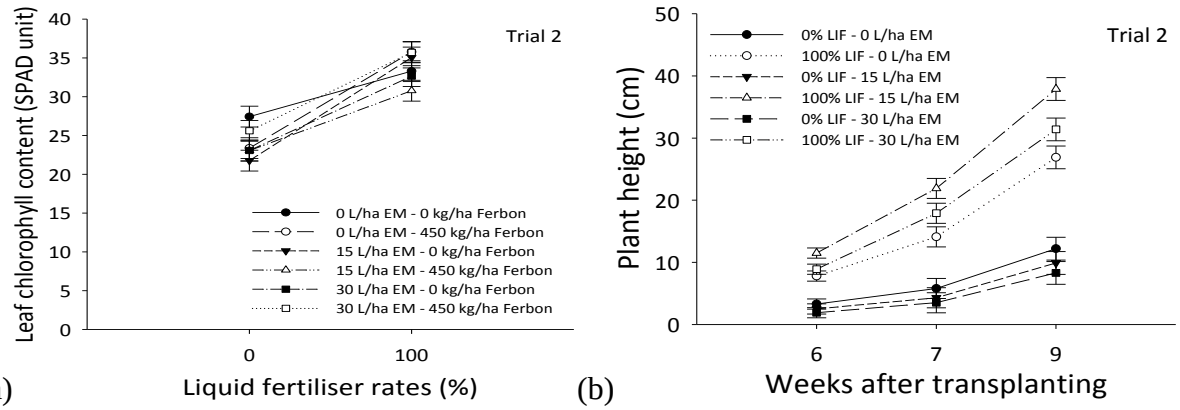


Figure 3.2 Interaction effects between (a) liquid inorganic fertiliser (LIF), effective microbes (EM) and Ferbon on chlorophyll content, and (b) LIF and EM on plant height of sunflower (*Helianthus annus L.* 'Dwarf Sunsation') in Trial 2.

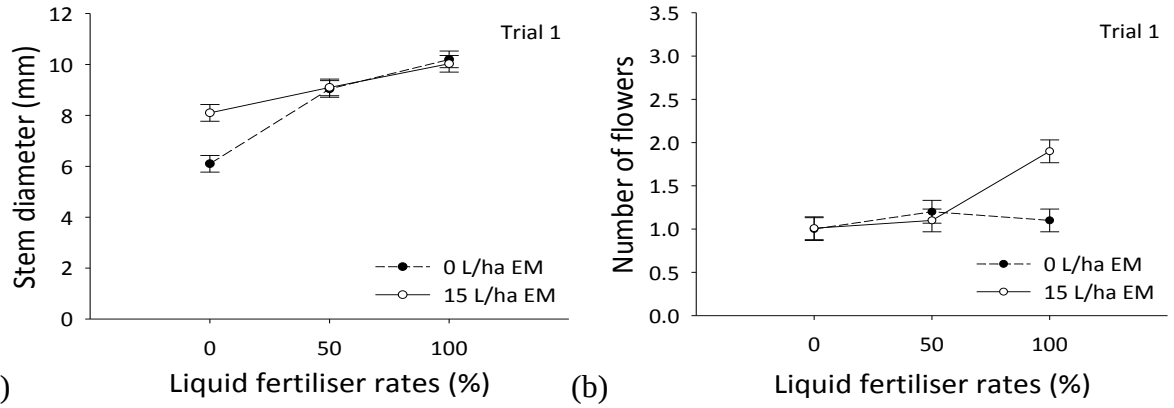


Figure 3.3 Interaction effects between liquid inorganic fertiliser (LIF) and effective microbes on (a) stem diameter, and (b) number of flowers in Trial 1 of sunflower (*Helianthus annus L.* 'Dwarf Sunsation').

Although node numbers were higher in the 15 L/ha EM treatments in Trial 1 (Table 3.1), Ferbon had no effect on node number in either trial. In both trials 100% LIF treatments resulted in a higher node number than the control (Table 3.1 & 3.2), 50% LIF treatments produced a significantly higher number of nodes.

The number of days until flowers appeared was reduced by EM inoculation in both trials (Table 3.1 & 3.2), but doubling the label rate to 30 L/ha had no effect compared with the control (Table 3.2). Application of 300 kg/ha Ferbon reduced time to flowering in Trial 1, but the lower rate of 150 kg/ha had no effect (Table 3.1). However, in Trial 2, Ferbon had no effect on flowering time (Table 3.2). Conversely, flowering time was reduced by LIF in Trial 2, but not in Trial 1 (Table 3.1 & 3.2).

The number of flowers per plant was significantly increased by EM treatment in Trial 1 (Table 3.1), but not in Trial 2 (Table 3.2). Ferbon treatment had no effect on flower number in Trial 1 (Table 3.1), but in Trial 2 it reduced the number of flowers per plant (Table 3.2). Flower numbers increased with increasing concentration of LIF in both trials. There was a significant positive effect between EM and LIF and flower numbers in Trial 1 (Figure 3.3b).

In both trials, flower head diameter was not affected by EM application (Table 3.1 & 3.2). The lower rate of 150 kg/ha Ferbon increased flower head diameter in Trial 1 compared both with the control and 300 kg/ha rate (Table 3.1); there was also an increase in flower head diameter in the 450 kg/ha treatments in Trial 2. Application of LIF significantly increased flower head diameter compared with the control, however there was no difference between the two concentrations (Table 3.1 & 3.2). Flower head diameter was significantly increased with applications of EM and LIF in Trial 1 (Figure 3.5a) and in Trial 2 (Figure 3.5b). Ferbon and LIF also produced a similar result (Figure 3.4).

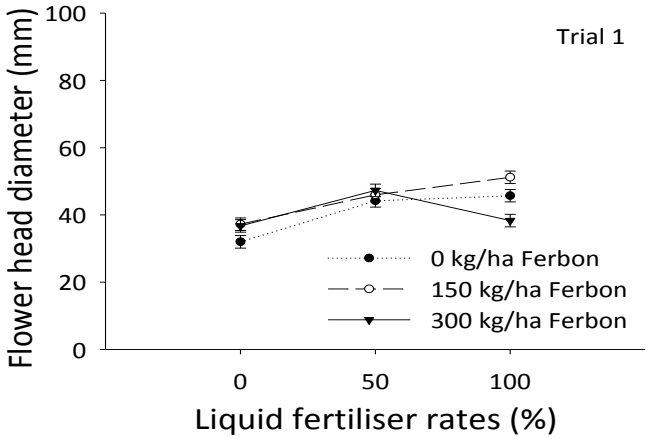


Figure 3.4 Effects of the interaction between liquid inorganic fertiliser and Ferbon rate on flower head diameter in sunflower (*Helianthus annus L.* 'Dwarf Sunsation').

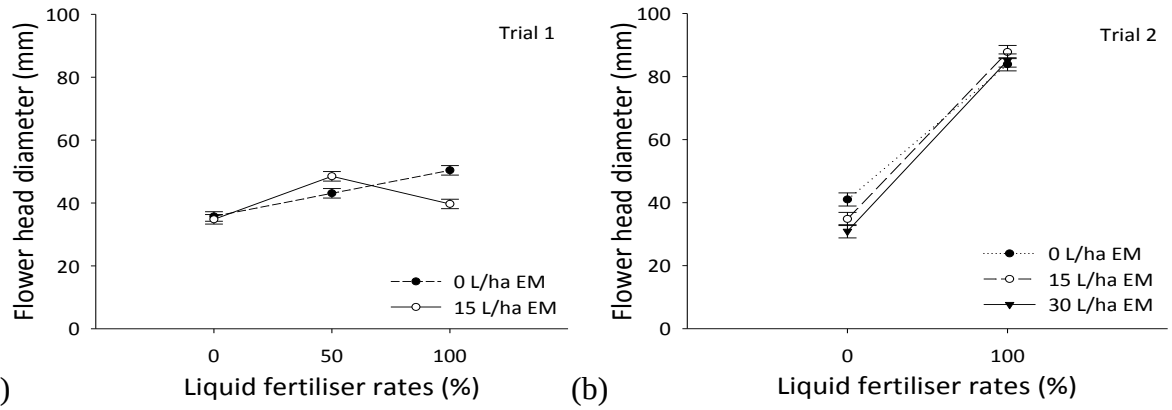


Figure 3.5 Interaction effects between liquid fertiliser and effective microbes on flower head diameter of sunflower (*Helianthus annus L.* 'Dwarf Sunsation') in (a) Trial 1 and (b) Trial 2.

3.5 Discussion

The application of EM in this study improved plant growth and hastened flowering. However, the reduction in plant height with the higher (double label) rate of EM suggests that the additional EM were utilising some of the nutrients, such as nitrogen, that would otherwise have been available for the plant. This is confirmed by the disappearance of the negative effect of EM in the presence of 100% LIF, and demonstrates the importance of ensuring adequate nutrient availability when applying bio-stimulants such as EM to the soil. This conclusion is supported by Yamada and Xu (2001) who reported that the success of EM inoculation to stimulate plant growth depends on the nutritional status of the soil. As well as their role in nutrient recycling, soil micro-organisms produce secretions of organic materials that mimic the effect of plant hormones (Higa and Parr, 1994). Hence, stimulation of plant growth through the use of bio-stimulants, combined with nutrient supplements, may not be due solely to an increase in available nutrients, but may, in part, be due to an increase in microbial activity. An imbalance between soil micro-organisms and available nutrients can also impact in other ways. For example, Breitenbeck *et al.* (1980) reported that micro-organisms contribute significantly to the loss of nitrogen through the process of nitrification within 2 to 3 weeks of addition.

While the addition of humate, in the form of Ferbon, did not impact on plant growth, the different interaction results between the two trials for LIF and Ferbon, and LIF and EM on flower head diameter and time of flowering may be due to the indirect effects of light exposure resulting from seasonal variation (spring versus summer) in day length between the two trials flower head diameter. The effect of day-length and light intensity on growth was also demonstrated by Friend *et al.* (1962). As mentioned previously, the effect of humate

substances on plant growth can depend on the length of the life cycle of the plant. According to Ertani *et al.* (2011), in the short term humates increase growth of plant roots, followed later by increased shoot and leaf growth. An increase in root growth means more surface area for nutrient uptake, enabling an increase in nutrient absorption, thereby often leading to an increase in total biomass of the plant. The addition of humate has been shown to increase the microbial biomass in the soil (Gumiński *et al.*, 1965). A low level of nutrients, combined with an abundance of micro-organisms in the rhizosphere, is likely to impact negatively on plant growth and development through competition for nutrients; hence, the balance between soil micro-organisms and nutrient levels is critical for optimising plant growth.

The reduction observed in leaf chlorophyll content may be a function of increased biomass, evidenced by increase in plant height and stem diameter, diluting nitrogen. According to Evans *et al.* (2000), most of the organic nitrogen in a protein form can be spread in different parts of the plant in order to further strengthen the process of photosynthesis, and this deployment could be related to the content of leaf chlorophyll. Ingestad (1979) also concluded that large distribution of nutrients can occur between leaves of different ages, leading to competition between leaves and thus reflecting on chlorophyll content.

3.6 Conclusion

The results of this study indicate that, in the presence of adequate nutrient availability, both effective microbes and lignite-based humates such as Ferbon have potential to increase plant productivity.

"Chapter 4" Effect of fertiliser type and mycorrhizal inoculation on growth and development of sunflower

4.1 Abstract

Biological farming practices using bio-inoculants and renewable organic supplements are being increasingly adopted by primary producers yet still little is known about their benefits in sunflower plants compared to conventional fertiliser practices. Two trials were conducted to compare the influence and interaction of bio-inoculation with arbuscular mycorrhizal fungi (AMF) with conventional inorganic and renewable organic fertilisers on the development and growth of sunflower plants. Commercially produced AMF was applied as a spore application with liquid organic fertiliser (LOF) applied at 0 and 50% in Trial 1; 0, 50% and 100% concentration of prepared label rate solution in Trial 2, or liquid inorganic fertiliser (LIF) applied at 0 or 100% concentration (Hoagland's solution regular strength with low P). Results showed limited interaction between AMF and fertiliser type. Sunflower plants inoculated with AMF and fertilised with LIF had greater plant height and stem diameter in Trial 1 and leaf chlorophyll content at various assessment times in both trials. The presence of hyphae and arbuscules increased in sunflower plants grown with AMF inoculation and LOF. When there were no interactions, there was a strong treatment influence of AMF inoculation on plant height in Trial 2, and number of nodes, flower head diameter, AMF colonisation and AMF structures in both trials. In addition, LIF (100%) increased the leaf chlorophyll content, number of nodes and flower head diameter in both trials, and flower number only in Trial 2. The commercial LOF had negligible influence on sunflower productivity but improved leaf nutrient status. Standard concentration of LIF (100%) improved sunflower productivity, and slightly improved leaf nutrient status compared to LOF. This study has demonstrated that while there were beneficial effects of AMF on plant growth, the use of LOF at the rates applied in this study did not benefit growth and in some cases restricted growth and flower production in sunflower plants.

Key words: organic, inorganic, fertiliser, mycorrhizae, elements, nitrogen, potassium, zinc.

4.2 Introduction

Most modern farming practices rely on the addition of inorganic fertiliser inputs to maintain or increase crop productivity, yet these are undesirable in alternative practices such as biological farming and prohibited in certified organic production (Kirchmann and Bergström, 2001, Hole *et al.*, 2005, Norton *et al.*, 2009). As inorganic fertilisers are a finite resource

(Cribb, 2010) and there is growing demand by consumers for sustainably produced crops, research into biological farming approaches to produce high yielding, high quality and nutrient rich produce is necessary. Yet, there is limited research investigating yield and productivity benefits on the growth of annual herbaceous crops using biological farming approaches such as bio-inoculants and renewable organic fertilisers compared to inorganic fertilisers (Treadwell *et al.*, 2007).

The use of bio-inoculants such as arbuscular mycorrhizal fungi (AMF) is of growing interest to primary producers implementing a biological approach to crop production (Wu *et al.*, 2005, Barrow, 2012). Arbuscular mycorrhizal fungi form beneficial associations with 80-92 % of recorded species of land plants (Schübler *et al.*, 2001, Wang and Qiu, 2006). Hyphae of AMF extend a considerable distance beyond the host plant roots, acting as carriers of important nutrients especially in weathered and nutrient depleted soil (Brundrett, 1996, Müller *et al.*, 2012).

For many species now widely cultivated, their wild types were likely to have exploited symbiosis with mycorrhizal fungi for nutritional benefits. Studies have demonstrated that AMF promote the absorption of phosphorous (P), nitrogen (N), iron (Fe), zinc (Zn) and copper (Cu) in switchgrass (*Panicum virgatum*), maize (*Zea mays*), cucumber (*Cucumis sativus*) and other host plants (Clark and Zeto, 2000, Lee and George, 2005, Perner *et al.*, 2006), and the previous elements plus sulphur (S), potassium (K), calcium (Ca) and magnesium (Mg) in switchgrass (Clark, 2002). In modified agricultural systems, research has shown that high levels of inorganic fertilisers (especially high N and P) can prevent effective AMF colonisation of plant roots that would naturally form mycorrhizal associations (Egerton-Warburton and Allen, 2000, Egerton-Warburton *et al.*, 2001, Adesemoye *et al.*, 2008). Consequently, the inability of plants to form mycorrhizal associations with AMF can lead to a decrease in plant canopy biomass and productivity (Koide, 1985). In organic or biodynamic production, AMF colonisation is encouraged through reduced inorganic fertiliser and restricted herbicide and fungicide use. There may be additional benefits to productivity from the use of renewable organic fertilisers and a positive interaction with AMF; however there has been limited research investigating this interaction.

Root colonisation by AMF has a major role in enhancing plant growth through improving plant absorption of non-mobile nutrients, especially P and Zn (Fernandez *et al.*, 2009) and sunflower has been a popular model study species. Chandrashekara *et al.* (1995) found that AMF inoculation significantly increased spore abundance, percentage of root colonisation,

total plant dry biomass, flowering time and maturity, and P content in sunflower tissue at later stages of the experiment. Koide (1985) working on sunflower plants concluded that sunflower leaf area was positively associated with the degree of AMF colonisation, while high soil P led to lower AMF colonisation of sunflower roots. The same effect was also noticed with high concentration of soil N.

Investigations into the use of organic products based on algae, seaweed residues and humates as renewable organic fertilisers are also increasing (Thirumaran *et al.*, 2009). Kumar and Sahoo (2011) demonstrated that products containing seaweed extracts can improve the physical, chemical and biological characteristics of the soil. Khan *et al.* (2009) showed that seaweed extracts improve the ability of the soil to retain water enabling increased nutrient exchange between plant roots and the rhizosphere, which also enhanced the activity of beneficial soil microbes.

Humic acid is commonly added to organic fertilisers and is derived from several sources such as marine algae or bacterial residues (Hatcher *et al.*, 1981) and coal deposits (Glaser *et al.*, 2002). Russo and Berlyn (1991) demonstrated that a blend of humic acid with marine algae, plant metabolites and B vitamins dramatically increased root biomass of red maple (*Acer rubrum*) seedlings. Humic acid has been shown to increase ion transport, P absorption, photosynthesis, respiration and oxygen absorption in maize plants (Russo and Berlyn, 1991), and elongation of roots and cells in bean seedlings (*Phaseolus vulgaris* L.) (Schnitzer and Poapst, 1967, Vaughan, 1974). Organic materials can enhance plant growth indirectly by stimulating the activity of other life forms in the soil, such as bacteria and fungi.

Sunflower is an important agricultural crop and with its ability to form symbiosis with AMF, provides opportunities to investigate different fertiliser strategies and their influence on mycorrhizal colonisation and ultimately sunflower productivity. The aim of this study was to investigate the productivity of sunflower plants with AMF inoculation using both a commercial renewable liquid organic fertiliser (LOF) and a liquid inorganic fertiliser (LIF). We hypothesised that AMF inoculation will increase leaf productivity (plant height, dry mass, flower number and size) and leaf chlorophyll content of sunflower with additional benefit derived from an interaction with the LOF. We also expected to see an increase in AMF colonisation of sunflower roots when fertilised with LOF compared to LIF leading to improved nutrient status and increased productivity.

4.3 Materials and Methods

4.3.1 Experimental design

Two pot trials were conducted under controlled glasshouse conditions at the University of Tasmania in Hobart, Tasmania. Trial 1 commenced in December 2013 and Trial 2 in September 2014, both concluding after 10 weeks. Seeds of sunflower (*Helianthus annuus* L., 'Dwarf Sunsation') were germinated in vermiculite. After two weeks, germinated seedlings were transferred to 16 cm pots containing 2.3 L of a basic potting mix (composted pine bark 80% by volume, coarse sand 20%, lime 3 kg/m^3 and dolomite 3 kg/m^3). Pots were placed on glasshouse benches (Figures 4.1a and 4.1b) and watered daily.

Figure 4.1 (a) Sunflower seedlings after transplanting (Trial 1), (b) sunflower plants at bloom stage (Trial 2).

For both trials, treatments were a factorial arrangement of AMF (plus/minus) and fertiliser type (organic/inorganic) (Table 4.1), established as a randomised complete block design with five replicates per treatment.

Table 4.1 Treatment applications.

	Trial 1		Trial 2	
Treatments	Fertiliser rates	AMF	Fertiliser rates	AMF
Control	0	-	0	-
AMF Only	0	+	0	+
LOF	50%	-	50%	-
	50%	+	50%	+
			100%	-
			100%	+
LIF	100%	-	100%	-
	100%	+	100%	+
LOF + LIF			50% + 100%	-
			50% + 100%	+
			100% + 100%	-
			100% + 100%	+

Mycorrhizal fungi were applied using the commercial spore preparation MYCORMAX™ (JH Biotech Australasia Pty Ltd). QuadShot® (SLTEC) was applied as the liquid organic fertiliser (LOF) and Hoagland's solution (Hoagland and Arnon, 1950, Foo *et al.*, 2013) applied as the liquid inorganic fertiliser (LIF). The components of each product are detailed in Tables 4.2 and 4.3.

Table 4.2 The compositional formula for the AMF application used in trials.

Treatment	Constituents
AMF (Mycormax™, 2010)	Active Ingredients 2%, Inert Ingredients 98% Glo*mus intraradices (Rhizophagous irregularis)* 46 CFU/gm, *Glomus mosseae* 19 CFU/gm. ectomycorrhizal fungi*: *Laccaria bicolor* 500 CFU/gm, *Pisolithus tinctorius* 15,300 CFU/gm, *Scleroderma cepa* 1,760 CFU/gm, *Scleroderma geastrum* 1,760 CFU/gm and *Scleroderma citrinum* 1,760 CFU/gm.

**Note that the ectomycorrhizal fungi Pisolithus tinctorius, Scleroderma cepa, Scleroderma geastrum, Scleroderma citrinum* and *Laccaria biocolorare would not have colonized sunflower. The asterids class (including the Asteraceae order, which is where sunflower is classified) are dominated by AM and ericoid mycorrhizal partnerships (Brundrett, 2009).*

Table 4.3 The compositional formula for fertiliser applications used in trials.

	N	P	K	S	Ca	Fe	Si	Mg	B
Quadshot (*100%) g/L (LOF)	0.032	0.136	0.192	0.016	0.008	0.0024	0.0088	NA	NA
Hoagland's solution g/L (LIF)	0.213	0.00155	0.197	0.064	0.5	0.00033	--	0.049	0.0005

	Zn	Cu	Mo	Mn	Fulvic acid	Humic acid	Fish emulsion	Kelp	Molasses
Quadshot (*100%) g/L (LOF)	NA	NA	NA	NA	0.02	0.504	0.616	0.616	0.616
Hoagland's solution g/L (LIF)	0.00005	0.00002	0.00001	0.0005	non	non	non	non	non

* LOF (100%): nutrient concentrations in 8 mL of fertiliser, which was diluted in 392 mL water.

For the pots receiving AMF treatments, 1.15 g Mycormax was mixed into the surface soil of each pot (equivalent to 500g/m^3) prior to seedling transplant. Fertiliser treatments were applied a week after transplanting. LOF was applied either at 4 ml/pot ([1]50% label rate equivalent to 20 L/ha) or 8 ml/pot (100% label rate equivalent to 40 L/ha). The LOF was applied weekly as a soil drench in 400 ml of water in each pot. The LIF treatment was applied as 400 ml/pot of Hoagland nutrient solution weekly. The control treatments received 400 ml/week of water.

4.3.2 Assessments

Plant height and number of nodes were recorded weekly. Flowering dates were recorded daily from the beginning of flowering until the last plant flowered at full bloom. The diameter of the primary flower head was measured and the number of axillary flowers counted. At the conclusion of each trial, the stem diameter was measured 2 cm above soil level. For both trials, leaf chlorophyll content was estimated weekly from five weeks post transplanting using a Minolta SPAD-502 meter. Four fully expanded mature leaves were selected on each plant at each assessment date.

[1] Literature pointed out that high concentration of humate substances can undesirably influence plant growth as humic acids can act like plant hormones Chen, Y. & Aviad, T. 1990. Effects of humic substances on plant growth. *Humic substances in soil and crop sciences: Selected readings***,** 161-186, Nardi, S., Pizzeghello, D., Muscolo, A. & Vianello, A. 2002. Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry*, 34**,** 1527-1536.. Since LOF contained high levels of humic acid and P compared to LIF, half the label recommendation rate (50%) of LOF was applied to avoid possible negative impacts. Nevertheless, sunflower performance negatively responded to this rate; therefore, the initial treatment of LOF (50%) was increased to 100% in the second Trial.

At harvest, the stem was cut at the soil surface and the fresh weight of stem, leaves and flowers were recorded. Below-ground structures were not included as they were used for mycorrhizal assessments. Plant material was then placed in individual paper bags, oven dried at 40 °C for three days and dry weight recorded.

Water content was defined as: Water content = Fresh weight – Dry weight

Percentage Dry matter content (DMC) was defined as:

$$\% \text{ DMC} = \frac{\text{dry weight}}{\text{fresh weight}} \; x \; 100$$

Leaf nutrient status of plants in Trial 2 was analysed by a commercial laboratory (CSBP Soil & Plant Analysis Laboratory, Western Australia).

4.3.3 Root colonisation of AMF

To confirm mycorrhizal colonisation in sunflower roots following treatment with AMF at harvest, fine roots were collected from the pots, washed with tap water, placed in 50% ethanol (v:v) and stored at 4° C. To prepare material for colonisation assessment, stored roots were rinsed with tap water, cut into 1–1.5 cm segments, placed in glass, screw cap 100 mL Schott bottles and covered with 5% potassium hydroxide (KOH). The Schott bottles were placed in a water bath at 80-90° C for 5-7 minutes. After heating, roots were strained and rinsed twice with tap water, rinsed in 3.5% hydrochloric acid (HCl) and returned to the rinsed Schott bottle. Root samples were stained with 5% black ink (Schaeffer) in lactic acid (Khaosaad *et al.*, 2006, Toussaint *et al.*, 2007) and reheated for 3 min. Roots were then strained, rinsed once with tap water and placed in water with a few drops of lactic acid to destain.

To assess AMF colonisation, the method of McGonigle *et al.* (1990) was modified using gridded slides. Five slides of five stained roots per slide mounted in water were prepared for each treatment. Using the modified gridded slide method, the crosshair eyepiece was replaced with gridded slides (twin grids 20 mm x 20 mm each with 1 mm line spacing) (Figure 4.2). AMF colonisation was scored by inspecting six intersections of the root with grid lines per root. As there were five roots per slide and five replicates per treatment, this gave a total of 150 intersections for each replicate per treatment (at each interaction of 150 intersections is counted the presence\absence each of hypha, vesicles and arbuscules). The following formula was used to calculate colonisation:

$$\text{Hypha (H), vesicle (V) or arbuscule (A) presence} = \frac{\text{X}}{150} x100$$

Where (X) = hypha, vesicle or arbuscule presence

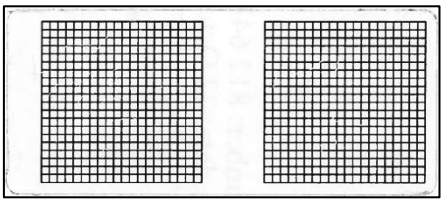


Figure 4.2 Gridded slide used to estimate AMF colonisation.

4.3.4 Data analysis

Data was analysed by analysis of variance (ANOVA) using R-3.2.2 software. A univariate general linear model approach was used with mycorrhizal inoculation and fertiliser treatment considered as fixed factors. For normality distribution, data of AMF colonisation were transformed into LOG (x + 1) only when necessary. Data are displayed as mean values for each treatment combination. Significance was calculated at $F\ prob \leq 0.05$ and least significant difference (LSD) used for comparison of treatment means in the tables and error bars on figures. Graphs were drawn using SigmaPlot 12.5 (Systat Software, Inc.).

4.4 Results

4.4.1 Canopy parameters

4.4.1.1 Plant height

There was a significant interaction (*P = 0.05, 0.002, <0.001, 0.003* and *0.07* for weeks 5, 6, 7, 8 and 9 respectively) between AMF and fertiliser type on plant height in Trial 1 at weeks 6, 7 and 8 (Figure 4.3a), attributed to the increase in height when plants were inoculated with AMF and treated with LIF. There was no interaction in Trial 2 at any assessment date, but a strong main effect of AMF inoculation was observed where sunflower plants were significantly taller at each assessment date (*P = 0.004, 0.001, 0.001, 0.007* for weeks 5, 6, 7 and 8 respectively) except in the final week, *P = 0.12* (Figure 4.3b).

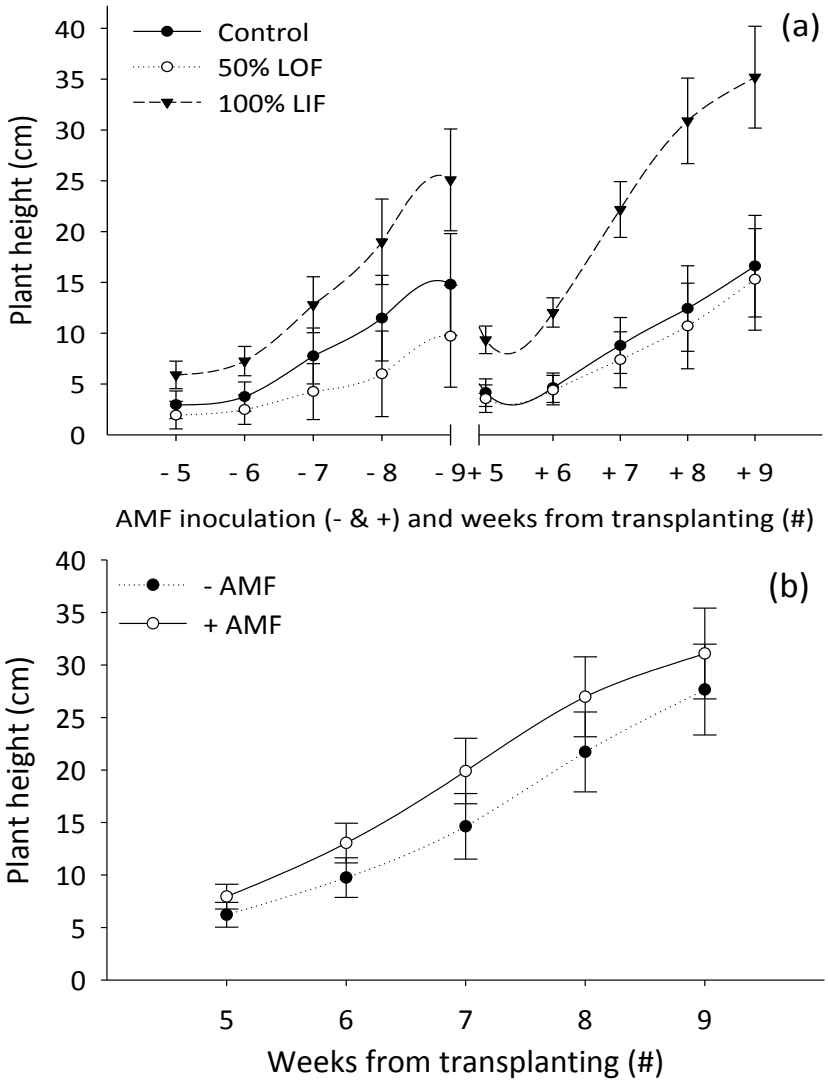
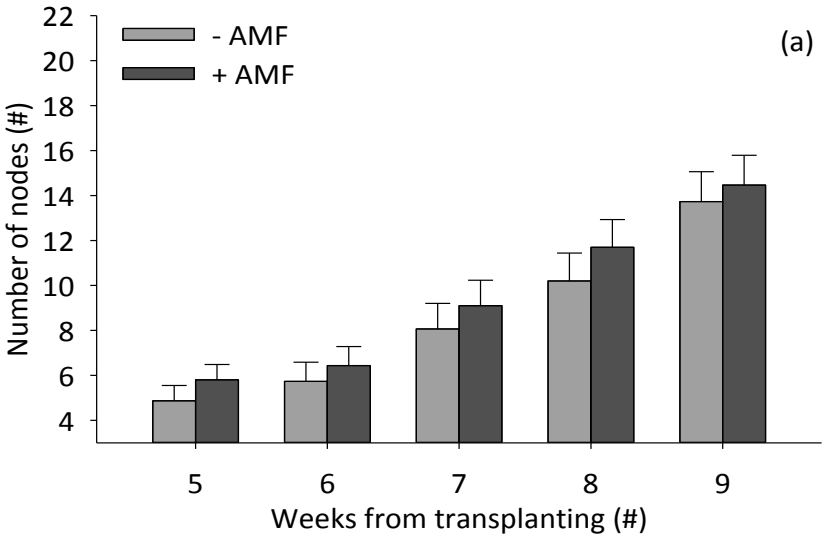
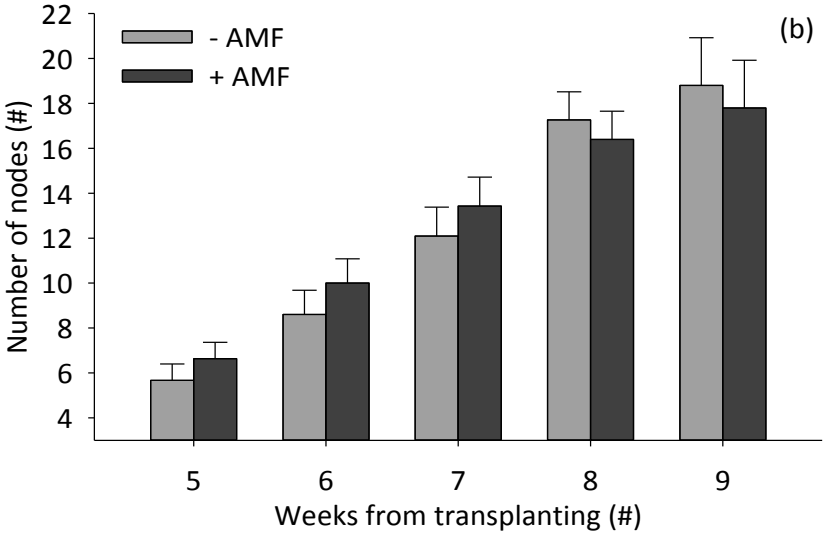


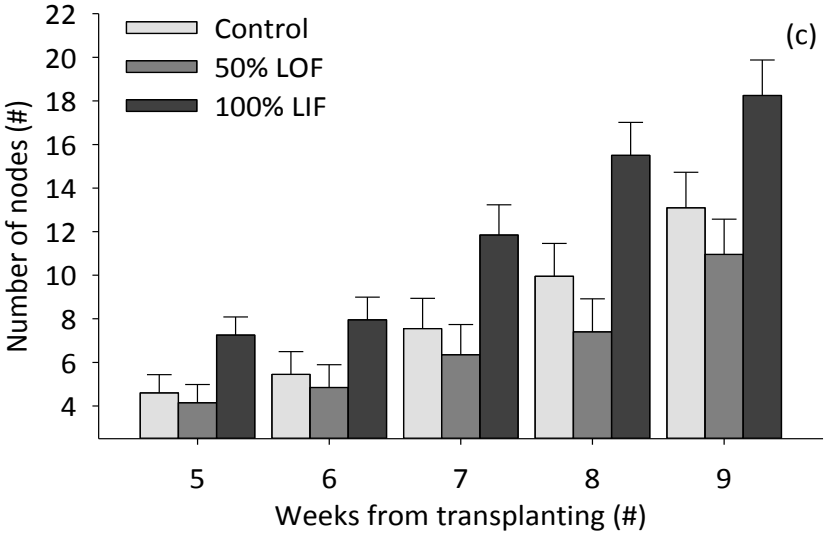
Figure 4.3 (a) Interaction between mycorrhizal inoculation (AMF) and fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on plant height, Trial 1. (b) Effects of mycorrhizal inoculation (AMF) on plant height, Trial 2.

4.4.1.2 Number of nodes

There was no interaction between AMF inoculation and fertiliser type on number of nodes in either trial (data not shown). Plants inoculated with AMF had significantly more nodes in weeks 5 and 8 (*P = 0.009* and *0.02* respectively) of Trial 1 (Figure 4.4a) and weeks 5, 6 and 7 (*P = 0.01*, *0.01* and *0.04* respectively) of Trial 2 (Figure 4.4b). Sunflower plants also had significantly more nodes under the LIF treatment in Trial 1 at each assessment date (P = *<0.001* for all weeks) with little difference between the control and 50% LOF treatment (Figure 4.4c). In Trial 2 node number was consistent for each treatment across trial except for week 8 where significantly more nodes were found in the 100% LIF treatment (data not shown).


- AMF
+ AMF
(a)
Number of nodes (#)
Weeks from transplanting (#)


- AMF
+ AMF
(b)
Number of nodes (#)
Weeks from transplanting (#)


Control
50% LOF
100% LIF
(c)
Number of nodes (#)
Weeks from transplanting (#)

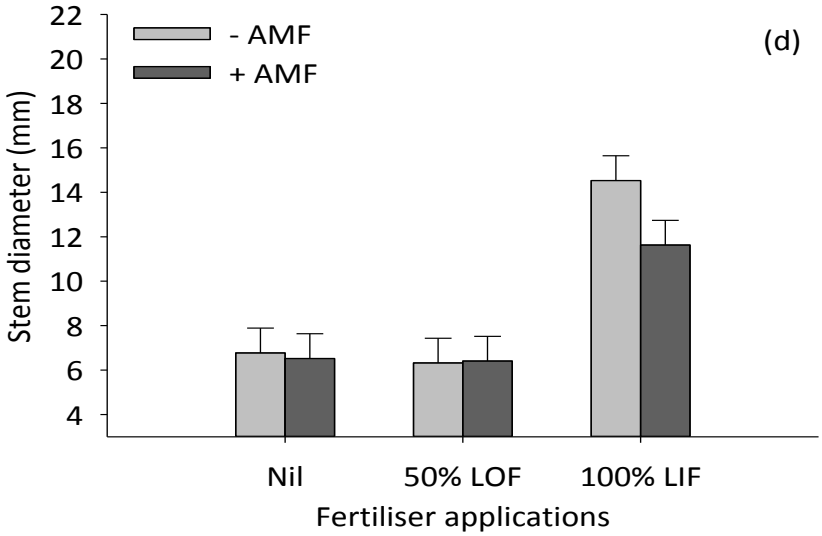


Figure 4.4 (a) and (b) Effects of arbuscular mycorrhizal inoculation (AMF) on number of nodes in Trial 1 and Trial 2, (c) fertiliser applications on number of nodes in Trial 1, and (d) interaction between AMF inoculation and fertiliser applications on stem diameter in Trial 1.

4.4.1.3 Stem and flower measurements

There were no interactions between AMF and fertiliser type on weight of dry matter and flower diameter, but stem diameter (Figure 4.4d) attributed to the influence of AMF inoculation on the LIF treatment which produced significantly wider stems in Trial 1 (*P = 0.001*). Inoculation of AMF increased primary flower head diameter in Trial 1 (Table 4.4) but reduced the time to flowering in Trial 2 (Table 4.5). LIF significantly increased stem diameter in Trial 2 (Table 4.5) and flower head diameter in both trials compared to each other treatment (Table 4.4 and 4.5). LOF (50%) increased flower time in Trial 1 compared with LIF but there were no differences between LOF and control treatment for flower time (Table 4.4); LOF had no effect on flower time in Trial 2 (Table 4.5). Flower number and weight of dry matter as measured in Trial 2 was higher in the LIF treatment and fertiliser type had no effect on DMC or water content (Table 4.5).

Table 4.4 Effects of (1) mycorrhizal inoculation (AMF) and (2) fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)] on flower diameter and flower time of sunflower, Trial 1.

Treatments	Flower diameter (mm)	Flower time (days)
(1) Mycorrhizal inoculation (AMF)		
- AMF	54.1 b	69.5
+ AMF	58.6 a	67.1
L.S.D.	*2.84*	*ns*
F prob.	*0.003*	*0.14*
(2) Fertiliser type		
Control	41.6 b	69.9 a
LOF (50%)	44.9 b	72.3 a
LOF (100%)	--	--
LIF	82.4 a	62.7 b
LOF (50%) + LIF	--	--
LOF (100%) + LIF	--	--
L.S.D.	*3.48*	*4.09*
F prob.	*<0.001*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 4.5 Effects of (1) mycorrhizal inoculation (AMF) and (2) fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on stem diameter, flower diameter, flower time, flower number, dry matter content (DMC) and weight of dry matter of sunflower, Trial 2.

Treatments	Stem diameter (mm)	Flower diameter (mm)	Flower time (days)	Flower number (#)	DMC (g/plant)	Dry weight (g)
(1) Mycorrhizal inoculation (AMF)						
- AMF	12.4	68.7	61.5 a	3.0	171	25.5
+ AMF	12.8	70.4	56.1 b	3.4	168	26.0
L.S.D.	*ns*	*ns*	*4.53*	*ns*	*ns*	*ns*
F prob.	*0.22*	*0.42*	*0.02*	*0.33*	*0.71*	*0.79*
(2) Fertiliser type						
Control	10.0 d	66.1 bc	59.1	2.4 c	159	17.5 c
LOF (50%)	11.4 c	62.8 c	56.4	2.4 c	156	15.7 c
LOF (100%)	11.0 cd	64.1 c	64.2	2.7 bc	173	20.1 c
LIF	16.5 a	79.3 a	61.5	4.8 a	181	40.7 a
LOF (50%) + LIF	13.0 b	73.1 ab	52.9	3.7 ab	165	29.2 b
LOF (100%) + LIF	13.8 b	72.0 b	58.7	3.3 bc	184	31.3 b
L.S.D.	*1.22*	*7.22*	*ns*	*1.29*	*ns*	*7.22*
F prob.	*<0.001*	*<0.001*	*0.09*	*0.003*	*0.31*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

4.4.2 Leaf chlorophyll content

A significant interaction between AMF and fertiliser type was observed on leaf chlorophyll content in Trial 1 in week 5 and week 6 only, driven largely by the 100% LIF treatment with

AMF at those dates (Figure 4.5a). There was no interaction in Trial 2 at any assessment date. AMF inoculation had no significant effect on leaf chlorophyll content in either trial (data not shown). While fertiliser type had a significant influence on leaf chlorophyll content, this effect was inconsistent between trials. In both trials, plants receiving LIF only had significantly higher leaf chlorophyll content at all assessment dates, with exception of Trial 1 week 7 and 8 (data not shown). In Trial 1, significantly lower leaf chlorophyll was observed in plants receiving the 50% LOF treatment in weeks 5, 7 and 8 (data not shown). In Trial 2, LOF (either alone or in combination with LIF) had a negligible effect on leaf chlorophyll content from the control (Figure 4.5b).

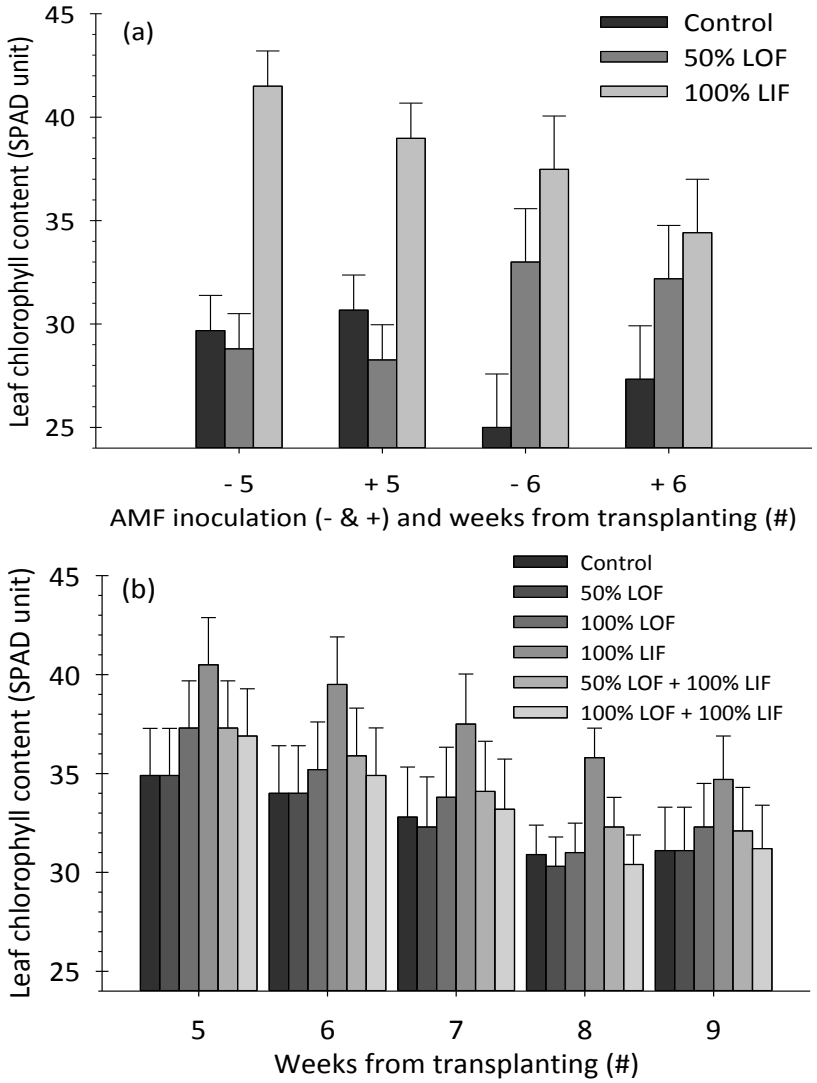


Figure 4.5 (a) Interaction between mycorrhizal inoculation (AMF) and fertiliser type (LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on leaf chlorophyll content in Trial 1, in Figure 3a, -5, +5, -6 and +6 mean week 5 and week 6; and -/+ symbols mean with and without AMF; (b) main effects of fertiliser types on leaf chlorophyll content Trial 2.

4.4.3 Leaf nutrient status

Significant interactions between AMF and fertiliser type were observed for P, K, B and Mn concentrations in sunflower leaves but not Total N, Ca, Mg and Zn (appendices Tables 9.1 and 9.2). It was difficult to identify a consistent driver of this variability. Inoculation with AMF plus LOF (100%) increased leaf P at $P = 0.03$ (Figure 4.6) and the LOF (50%) plus LIF treatment with AMF inoculation increased K and B concentrations (appendices Tables 9.1 and 9.2). In contrast, a significant increase in Mn concentration was observed for leaves treated with LOF (100%) minus AMF inoculation (Appendices Table 9.2).

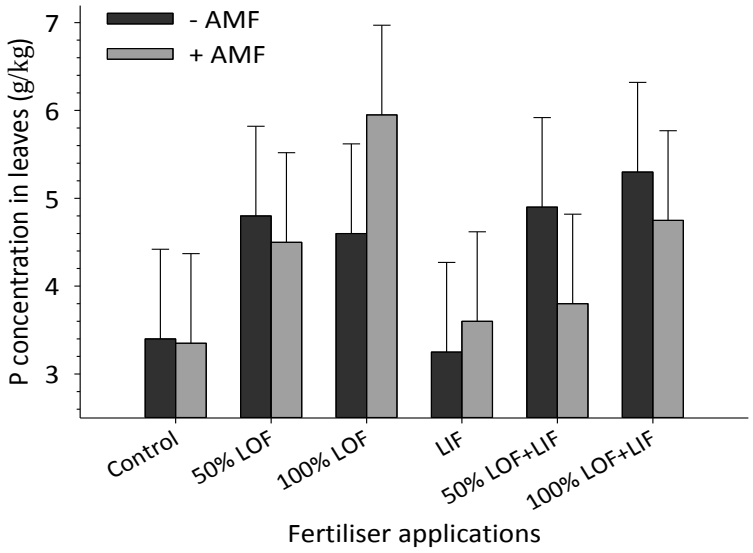


Figure 4.6 Interaction between mycorrhizal inoculation (AMF) and fertiliser type (LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on leaf phosphorus (P) concentration.

Leaf concentrations of Total N, Zn, Ca and Mg were unaffected by AMF inoculation (Appendices, Table 9.3). A significant effect was noticed on total N concentration in leaves with fertiliser treatments, but the effect was not consistent (Appendices, Table 9.3). LOF rates increased leaf Mn and Zn compared to other treatments (Appendices, Table 9.3), and increased leaf Ca, but there were no differences between LOF rates and control treatment on leaf Ca (Appendices, Table 9.3). Fertiliser applications had no effect on leaf Mg (Appendices, Table 9.3).

4.4.4 Mycorrhizal colonisation

Hyphae, vesicles and arbuscules were found in both inoculated and non-inoculated roots in Trial 1 (Figure 4.7). In Trial 2, only colonisation structures in AMF inoculated plant roots were observed.

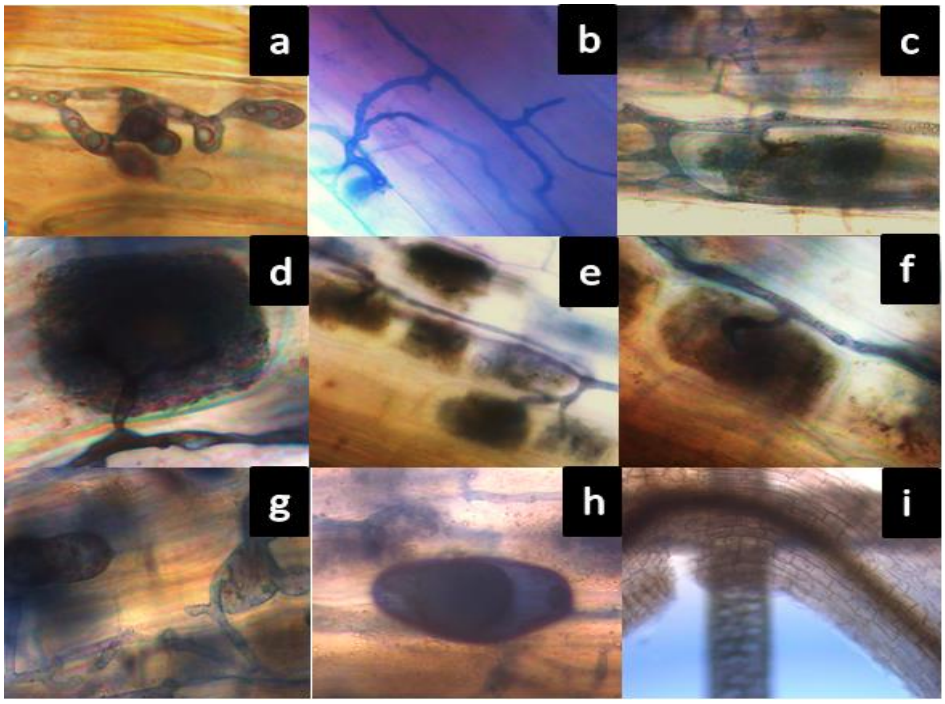


Figure 4.7 a) Stained hyphae (control treatment) inspected by X400 lens. b) Stained hyphae (AMF treatment) inspected by X200 lens. c) Stained arbuscules and hyphae (50% LOF) inspected by X400 lens. d) Stained arbuscules (LIF treatment) inspected by oil lens X100. e) Stained arbuscules and hyphae (50% LOF + AMF) inspected by X400 lens. f) Arbuscules within root cell linked to hypha (LIF + AMF treatment) inspected by X400 lens. G) Hyphae and vesicles (100 % LOF treatment) inspected by X400 lens. h) Vesicle (AMF + 100% LOF) inspected by X400 lens. i) Clean root placed on gridded slide (50% LOF treatment), inspected by X200 lens.

Significant interactions between AMF and fertiliser type were observed on hyphae and arbuscules in Trial 1, and arbuscules in Trial 2. More hyphae and arbuscules were observed when plants were inoculated with AMF and fed LOF (50%) in Trial 1 (Figure 4.8 a and b respectively), and more arbuscules were observed when plants were also inoculated with AMF and fed LOF (100%) or LOF (50%) plus LIF (Figure 4.8 c and d) in Trial 2.

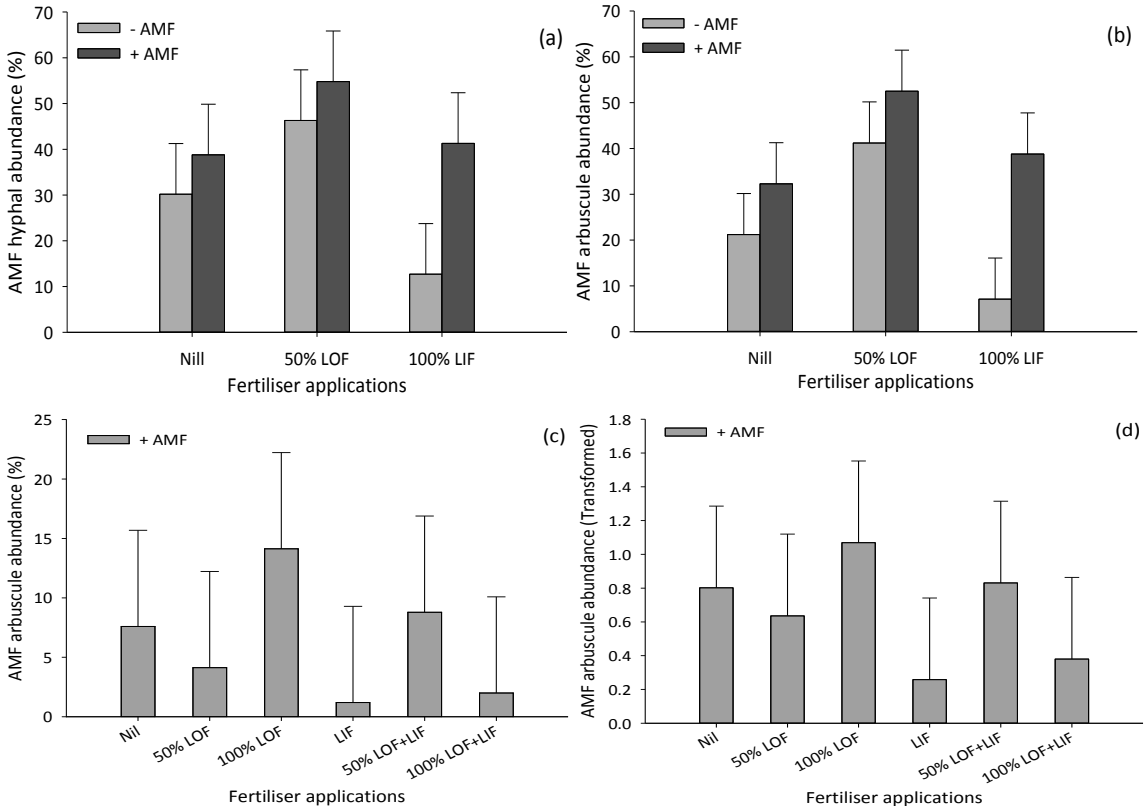


Figure 4.8 Interaction between mycorrhizal inoculation (AMF) and fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)] on the presence of (a) mycorrhizal hyphae, (b) arbuscule in Trial 1, and (c (normal data)and d (transformed data)) in arbuscule in Trial 2 in roots of sunflower plant (*Helianthus annuus* L., 'Dwarf Sunsation').

Inoculation by AMF increased the abundance of hyphae and arbuscules in Trial 1 (Figure 4.9 a). Mycorrhizal colonisation was not detected in uninoculated plant roots in Trial 2 compared to the inoculated plant roots. Treatment with LOF increased the abundance of hyphae and arbuscules in Trial 1, and arbuscules in Trial 2. Colonisation was significantly higher in the LOF treatments compared with the LIF treatments in trial 1 (Figure 4.9 b). In Trial 2 (Figure 4.9 c and d), the abundance of arbuscules was higher in the 100% LOF compared with 100% LIF. In general, LIF decreased or had no effect on AMF structures in both Trials (Figure 4.9 b, c and d). Compared with the unfertilised controls, both LOF and LIF had no effect on hyphae or vesicle abundance in Trial 2 (Figure 4.9 c), but the abundance of vesicles was reduced when plant fed with LIF in Trial 1 (Figure 4.9 b).

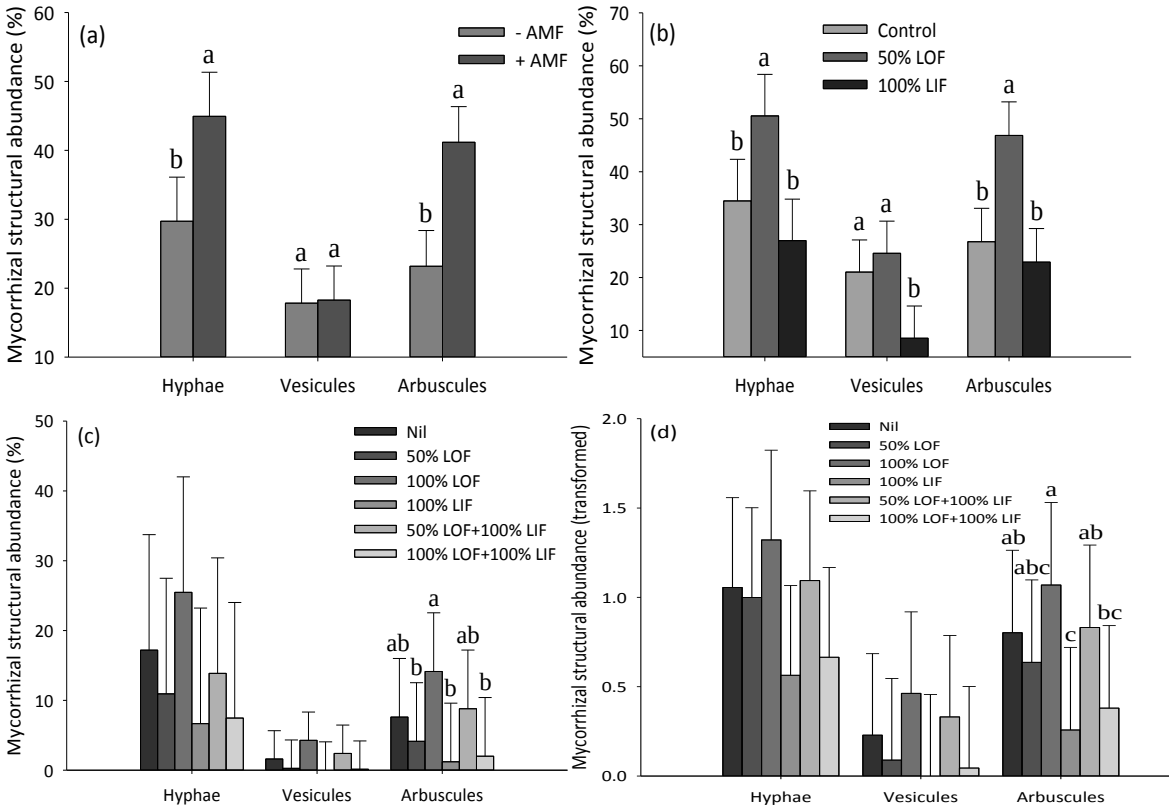


Figure 4.9 The presence of AMF colonisation structures in sunflower roots following AMF inoculation (a), LOF and LIF fertiliser applications (b) in Trial 1; and LOF and LIF fertiliser applications (c and d (transformed data)) in Trial 2.

4.5 Discussion

Biological approaches to agricultural production have the potential to sustainably increase crop productivity through practices such as inoculating soil with beneficial mycorrhizal fungi which can better utilise available nutrients. Whilst research has demonstrated the positive influence of AMF on the productivity of some crops (Chandrashekara *et al.*, 1995, Kaya *et al.*, 2003, Patra *et al.*, 2012), still little is known about the interactions between AMF and different fertiliser types on crop productivity. This study has demonstrated mixed results on sunflower productivity following inoculation with AMF and fertiliser treatments. There was limited interaction between AMF and fertiliser type and often results were unexpected.

4.5.1 Growth parameters of sunflower

Sunflower plants inoculated with mycorrhizal fungi and fertilised with inorganic fertiliser increased plant height and stem diameter in Trial 1 and leaf chlorophyll content at various assessment times in both trials. When there were no interactions, there was a strong main effect of AMF inoculation on number of nodes and flower head diameter in both trials and plant height in Trial 2 compared to no inoculation. LIF was the strongest performing fertiliser

treatment increasing the number of nodes and flower head diameter in both trials, and flower number which was only recorded in Trial 2. The commercial LOF formulation had negligible influence on sunflower productivity but improved the leaf nutrient status. In contrast, whilst standard concentration of LIF improved sunflower productivity it only slightly improved leaf nutrient status compared to LOF.

The improvement of sunflower productivity in general with LIF alone or plus AMF treatment may be due to the amount of N added by LIF compared to LOF. Metabolic processes and the increase in the canopy growth and yield rely on the supply of N fertiliser (Lawlor, 2002, Cechin and de Fátima Fumis, 2004). Although leaf chemical composition did not show differences in leaf N-content between LIF and LOF treatments, plants that received the standard rate of LIF accumulated more N than LOF plants due to their increased biomass. Several studies have demonstrated that the rapid growth of many plant species in fertile soils leads to the reduction in the concentration of nutrients in their tissues as a result of diffusion and dilution of these nutrients in plant tissues (Boyd and Hess, 1970, Harner and Harper, 1973, Auclair, 1977, El-Ghonemy *et al.*, 1978, Williams *et al.*, 1978).

Compared to LOF applications, an increase in stem height and diameter of sunflower plants occurred with the interaction between AMF inoculation and standard LIF application. This was unexpected given that reduced mycorrhizal colonisation was observed when sunflower plants were fertilised with LIF. However, the interaction was driven largely by the strong influence of LIF fertiliser on plant growth. Furthermore, when there were no interactions between AMF and LIF, flower head diameter significantly responded with LIF application. This may also be due to the effect of the amount of N added by LIF. Massey (1971) found that sunflower height increased 11 cm compared to control when plants were fertilised with 56 kg N ha^{-1}, while stem diameter was significantly influenced by plant spacing instead of N fertiliser. Ozer *et al.* (2004) and Ayub *et al.* (1998) found different rates of N increased stem height and diameter of sunflower. Hence, it can be deduced that N is a key player in the growth and development of the plant biomass; therefore, the limited impact of LOF applications may be due to the small amount of N added, compared to the LIF application.

Sunflower height and flower head diameter were increased through AMF colonisation (as a main effect) which resulted in improved uptake of other nutrients such as B. An increase in leaf B was observed in plants receiving AMF. Asad *et al.* (2003) and Asad (2002) suggested that sunflower is highly sensitive to low B supply where deficiencies reduce vegetative growth and the development of reproductive organs. McIlrath and Skok (1964) also reported

that sunflower height and internode length significantly increased in plants that received B nutrition compared to control plants which received no B and Farokhi *et al.* (2014) found that B nutrition increased flower head diameter of sunflower plant. Further studies are required to confirm whether improved B nutrition is responsible for improved sunflower productivity under different levels of N nutrition.

4.5.2 Leaf chlorophyll content

AMF inoculation plus an adequate amount of N increased leaf chlorophyll content in sunflower plants. A significant interaction between AMF inoculation and LIF application influenced leaf chlorophyll content; and a decrease in leaf chlorophyll content was observed in non-inoculated plants. This may be due to the rivalry between plants and AMF on the amount of available-N. Therefore, if AMF promote the acquisition and delivery of N to the inoculated plants, as observed in the interaction between AMF inoculation plus an adequate amount of N. Under low N conditions, even small amounts of added N may confer the plant a competitive advantage with symbionts on the available N (Hodge and Storer, 2015).

Where there were no interactions (i.e. Trial 2), leaf chlorophyll content was strongly influenced by the standard LIF application. The standard LIF application was the strongest performing fertiliser treatment, increasing the leaf chlorophyll content in sunflower leaves by almost 10%. Over 80% more N is applied in the LIF treatments than the LOF treatment, thus the possibility of a variation in the leaf content chlorophyll is great with this amount of N. Cechin and de Fátima Fumis (2004) observed that chloroplasts contribute as much as 75% of the leaf-N and attributed low levels of chlorophyll to declining photosynthetic processes under conditions of limited N.

With the low amount of N added by 50% LOF application in this study (Trial 1), the results showed that AMF inoculation with LOF application improved leaf chlorophyll content. This is probably due to the improved AMF inoculation which influenced the uptake of other nutrients, such as P which plays an essential role in the synthesis of chlorophyll. In trial 2, leaf-P concentrations improved with 100% LOF application plus AMF. The results also indicate that all AMF colonisation structures responded significantly to the application of 50% LOF, which can lead to a significant increase in the P content of the leaf. Plesničar *et al.* (1994) observed a decrease in the leaf chlorophyll content in sunflower plant under P-deficiency.

4.5.3 AMF colonisation

LOF treatments with AMF inoculation improved the abundance of hyphae and arbuscules in sunflower roots (i.e. Trial 1) while no mycorrhizal colonisation was detected when LIF was applied in Trial 2. This is likely due to the addition of spores by the commercial inoculum and the benefit of humic acid in the LOF product. Humic substances have been shown to improve AMF colonisation. For example, working on hydroponics, Gryndler *et al.* (2005) observed that humic substances (humic and fulvic acids) stimulated AMF colonisation on maize roots in a hydroponic culture, and further suggested that humic substances may also stimulate AMF colonisation in the soil environments.

Studies demonstrated that chemical fertilisers containing high concentrations of P led to low AMF colonisation and reduced the symbiotic associations between host plants and AMF (Verkade and Hamilton, 1983, Koide, 1985, Thompson, 1987, Johnson, 2010). Although LIF contained low P, it had high N which played an important role in the reduction of AMF colonisation for treatments that received the standard rate of LIF application. Nutrition by fertilisers rich in N are sometimes indirectly specified to the mutual benefit and the formation of the symbiotic associations between the host plant and AMF. Mäder *et al.* (2000) mentioned that intensive agriculture or nutrient enrichment by N and P inputs reduce benefits especially from the mycorrhizal symbiosis, to increase the ability to take up nutrients from the soil. Mäder *et al.* (2000) reported that host plants exposed to high nutrient levels severely reduced or stopped providing their fungal partners with sources that they need and in turn reduced mycorrhizal colonisation. Therefore, the reduction in AMF colonisation in LIF plants may be due to the abundance of nutrients thus making the symbiotic associations with AMF irrelevant.

Although mycorrhizal colonisation increased with LOF treatments, these did not provide sufficient fertility for sunflower productivity when compared to the LIF fertiliser which provided all the necessary nutrients for plant growth.

4.6 Conclusion

The results of this study show that the symbiotic relationship between plant roots and AMF can have moderate impacts on leaf chlorophyll content in the absence of sufficient nutrients that contribute in one way or another in the synthesis of chlorophyll and in the same circumstances can enhance the apical growth of plants. The results confirmed that commercial organic fertilisers based on humic substances and seaweed extracts do not always

have positive effects on leaf chlorophyll and plant growth attributes such as plant height, number of nodes and flower number and size compared to inorganic fertilisers. This is likely due to the lower concentration of some nutrients in these fertilisers, or the materials used in the preparation of these fertilisers. Additionally, the application type whether in combination or alone may be influential. Finally, our findings indicate that AMF colonisation of sunflower roots improved with the addition of organic fertilisers based on humic substances (LOF) compared to inorganic fertiliser. Although this increase in colonisation did not increase plant growth, this lack of effect may be due to nutrient concentrations that were insufficient in the rates of LOF applied, compared to the higher concentrations applied with the LIF. Thus, the effect of improvement in AMF colonisation has not been confirmed. However, it was shown that mycorrhizal colonisation decreased with LIF treatments. Future research is required to examine AMF colonisation and its effect on plant growth attributes under higher concentrations of nutrients in the organic fertiliser based on humic substances and seaweed extracts with different concentration of mineral nutrients such as nitrogen sources, different species of plants and different types of soil to confirm where the positive and negative effects come from.

"Chapter 5" Effects of organic supplements, AMF inoculation and soil type on sunflower growth

5.1 Abstract

The effect of arbuscular mycorrhizal fungi (AMF, commercial product: MYCORMAX®) or organic supplements (Ferbon® - a lignite-based soil conditioner, compost and soluble humate granules™ (SHG)) on sunflower growth was examined. Plants were pot-grown in glasshouse conditions in two soil types, a clay loam sourced from an undisturbed forest site and a sandy loam from an orchard. Initial levels of total and available nutrients were higher in the forest soil than the orchard soil, as was organic carbon (5.15% compared with 0.53%). AMF inoculation led to significant effects on nutrient levels, with lower levels of NH_4^+-N, NO_3^--N, P, K, Mn and Zn in AMF inoculated forest soil compared with non-inoculated. In the orchard soil, most nutrients were higher in AMF inoculated soils compared with non-inoculated. Soil pH increased in AMF inoculated soils; SHG reduced pH in the forest soil but both compost and SHF increased pH in the orchard soil. AMF inoculation and organic supplements enhanced AMF colonisation in both soils. All colonisation structures significantly increased with AMF inoculation, except vesicle abundance in the forest soil. Ferbon and compost resulted in better AMF colonisation in the forest soil, while compost additions alone or combined with SHG resulted in better AMF colonisation in the orchard soil. There were strong treatment effects of compost on plant height and SHG on stem diameter in forest soil. In the orchard soil, primary flower head diameter increased by 22% and 24% with Ferbon and SHG respectively, but the same treatments reduced flower head diameter in the forest soil. Flower head diameter was significantly increased in AMF inoculated plants in orchard soil. The results of this study showed that the improvement of sunflower growth is possible with AMF inoculation under both low and high-nutrient soils. Nutrient availability in both soil types was strongly influenced by the organic supplements, especially humic supplements. In comparison with compost, humic supplements combined with AMF inoculation may not directly impact plant productivity under excessive presence of nutrients; however they can enhance AMF colonisation, the status of foliar nutrients and the health and fertility of the soil by regulating the release of nutrients.

Key words: Ferbon, growth, compost, nutrient, mycorrhizae, soluble humate.

5.2 Introduction

Inorganic fertilisers have been widely used to correct soil nutrient imbalances and promote productivity of many crop types (Reganold *et al.*, 1993, Welbaum *et al.*, 2004, Araújo *et al.*, 2009). These fertilisers add nutrients in plant-available forms and exact amounts are relatively easy to apply in various forms (e.g. granulated products or via fertigation). However, some researchers have argued that the disadvantage of reliance on inorganic fertilisers alone for nutrient management is a decrease in soil organic matter leading to degradation of soil quality (Reganold *et al.*, 1990, Reganold *et al.*, 1993, Glover *et al.*, 2000, Wells *et al.*, 2000). In addition, leaching of available nutrients and the finite nature of these resources are limitations to sustainable future use (Reganold *et al.*, 1993, Welbaum *et al.*, 2004, Major *et al.*, 2009).

Organic amendments such as compost and cover crop residues to promote plant growth have become increasingly popular in agricultural systems (Quilty and Cattle, 2011). Compost is the most common organic soil amendment (Quilty and Cattle, 2011) and it serves as an important source of nutrients, increases soil carbon stocks and improves both soil structure and water retention (Cavagnaro, 2014). Despite the benefits, solid organic amendments are physically difficult to apply on a large-scale and ready access is not always possible (Baziramakenga and Simard, 2001, Mkhabela and Warman, 2005, Risse and Faucette, 2009, Baldi *et al.*, 2010). Composts have a variable nutrient profile depending on the parent materials and nutrients are often not readily plant-available (Mkhabela and Warman, 2005, Lakhdar *et al.*, 2009). Compost can fail to provide plants with some essential nutrients such as potassium (Barker *et al.*, 1997, Baziramakenga and Simard, 2001).

An alternative to both inorganic fertilisers and compost amendments are ready sources of humic substances. Humates are the result of the decomposition of organic biomass (including plant and animal residues) in the soil (MacCarthy, 2001, Rose *et al.*, 2013, Rose *et al.*, 2014). Humic substances are present in the soil naturally, but the levels of these substances vary based on horticultural practices such as tillage which lead to a decline in levels of organic biomass and therefore humic substances (Novotny *et al.*, 1999, Shepherd *et al.*, 2001). Humic substances increase the absorption of nutrients and cell permeability (Russo and Berlyn, 1991). Humic acids have been shown to facilitate the conversion of some nutrients into their plant-available forms and can also act as plant hormones (Ouni *et al.*, 2014). For example, Muscolo *et al.* (2007) observed that the humic fraction resulted in an increase in growth of carrot cells similar to that caused by promoted morphological changes and 2,4-

dichlorophenoxyacetic acid similar to those induced by IAA. Chen and Aviad (1990) also found that plant growth increased with increasing humic substances in nutrient solutions; however they observed a decline in growth at very high concentrations. Early studies by Chen and Solovitch (1988) suggested that the optimum concentrations of humic acid range between 50 to 500 mg L^{-1}.

Commercial humate products are now widely available, including solid humate granules and bio-humate soil conditioners (e.g. Ferbon®). Studies confirming the benefits of modifying the soil with humic substances have been reported, including improvement of soil structure and aggregation, increasing water retention capacity, pH buffering, soil capacity to hold nutrients, bioavailability of immobile nutrients and cation exchange capacity (Rose *et al.*, 2013). Humic substances such as humic and fulvic acids are becoming widespread as commercial supplements for crop improvement but the effects of these substances can be difficult to predict (Rose *et al.*, 2013, Brown *et al.*, 2014). There are few objective studies which compare the effect of humate amendments on plant growth, either in comparison to or in combination with compost. This study seeks to make that comparison using sunflower (*Helianthus annuus* L.) as model crop system.

The growth and development of sunflower has been shown to increase significantly in the presence of mycorrhizal colonisation, due to enhancement in nutrient uptake (Brundrett, 2002), particularly P (Chandrashekara *et al.*, 1995, Ultra Jr *et al.*, 2007) under field conditions and in rhizoboxes. Arbuscular mycorrhizal fungi (AMF) are involved in beneficial symbioses with host plant roots of most food crops (Smith and Smith, 2002, Smith and Read, 2008) and the benefits are particularly prominent in soil with low nutrient availability (Douds and Schenck, 1990). Perner *et al.* (2006) reported that root colonisation by AMF often results in increased absorption of P, N, Cu, Zn and sometimes K. Given these benefits, it is important to understand how different organic supplements influence AMF colonisation.

This study investigated the growth and performance of sunflower plants grown in two different soil types of contrasting nutrient concentrations with treatments of AMF and organic amendments. This information will better support an understanding of when application of such amendments may provide a benefit to crop production and when they may not.

Specific questions addressed by the study include:

- How does compost application compare to alternative organic amendment products which are easier to apply (i.e. Ferbon® or soluble humate granules (SHG))
- Does soil nutrient status affect whether compost, Ferbon or SHG better support plant growth?
- Does the presence of AMF determine whether compost, Ferbon or SHG better support plant growth?

5.3 Materials and Methods

To evaluate the effect of mycorrhizal symbiosis and organic supplements on growth and development of the plant, a trial was carried out under glasshouse conditions, located in Tasmania Institute of Agriculture, Hobart, Australia. Seeds of sunflower plant (*Helianthus annuus* L. 'Dwarf Sunsation') were used in this trial. The trial was conducted between October 30, 2014 and January 2015.

5.3.1 Soil collection

Two types of soil collected at a depth of 0 to 20 cm were used. The first soil (clay loam) was collected from an undisturbed dry sclerophyll eucalypt forest in Sandy Bay, Tasmania (latitude -42.906778, longitude = 147.321868). The second soil (sandy loam) was collected from the headland of a commercial orchard in Lucaston, Tasmania (latitude -42.994256, 147.059139). Before the establishment of the trial, particle and chemical analysis were undertaken for both soils. The analysis for both soil types is described in Table 5.1.

Table 5.1 Chemical and particle size analysis of the soils before planting.

	NH_4^+-N (mg/kg)	NO_3^- - N (mg/kg)	P (mg/kg)	Total K (mg/kg)	S (mg/kg)	Exc. Ca (meq/100 g)	Exc. Mg (meq/100 g)	Exc. K (meq/100 g)	Exc. Al (meq/100 g)	Exc. Na (meq/100 g)
Forest	57.0	51.0	18.00.0	199.0	12.0	21.1	9.4	0.48	0.07	0.61
Orchard	5.0	11.0	7.0	30.0	62.8	0.77	0.78	0.08	0.06	0.28
	Fe (mg/kg)	**B (mg/kg)**	**Mn (mg/kg)**	**Zn (mg/kg)**	**Cu (mg/kg)**	**pH (CaCl2)**	**pH (H_2O)**	**Org. C (%)**	**Texture**	**Conductivity (ds/m)**
Forest	177.5	1.4	125.5	30.3	2.8	5.0	5.7	5.2	2.0	0.21
Orchard-	33.9	0.40	1.30.0	0.63	0.49	5.4	5.9	0.53	1.0	0.12
	Particle size analysis of the soil									
	Clay %		**Coarse Sand (%)**		**Fine Sand (%)**		**Sand (%)**		**Silt (%)**	
Forest	28.7		16.7		30.3		47.0		24.4	
Orchard	10.4		38.5		34.3		72.8		16.8	

5.3.2 Trial establishment

Pots of 16 cm diameter were filled with approximately 2 L of soil. The soil was mixed with controlled-release fertiliser in the form of Osmocote (11-4.8-14.9 NPK + trace elements) added to the soil prior to potting at the rate of 7.5 g per pot. Seeds of sunflower (*Helianthus annuus* L., 'Dwarf Sunsation') were germinated in vermiculite. The seedlings were transplanted into pots after emergence of the cotyledons (two weeks old) and were irrigated daily.

Trial design was a randomised complete block design with three factors. The trial had five replicate blocks with each replicate block containing 20 plants, divided into two groups of ten plants each. The first 10 plants (not inoculated) were sub-divided into 2 groups of five plants each for the orchard and the forest soils. The second set of 10 plants were also divided into two groups of five plants each and inoculated with AMF. The plants were then arranged randomly within the blocks and each block placed onto a different bench in the glasshouse. The factors were soil type (forest, orchard), AMF application (plus, minus) and organic supplement type (four types and a control). Details of the organic supplements are provided below and nutrient composition is described in Table 5.2.

- Control: control treatment – no supplements added
- Ferbon® (FF50SB, a lignite-based soil conditioner) at 0.6 g/pot (300 kg/ha), manufactured by: Interstate Energy Group Pty Ltd, Bacchus Marsh Victoria Australia.
- Compost at 1.6 g/pot equivalent to 800 kg/ha, (Foundation aerobic compost, Pure Living Soil Pty Ltd).
- Soluble humate granules (SHG) at 0.044 g/pot (22 kg/ha), (75% water soluble potassium humate with solubility of 85% and particle size 0.5 - 5 mm, Nutri Tech Solutions®).
- Compost plus SHG at the same rates given above.

The organic supplements were added to the pots two weeks after transplanting. The Ferbon and compost were gently mixed into the top centimetre; the SHG were dissolved in 400 ml water, then added to the pots as a soil drench. The AMF product used was MYCORMAX (Arbuscular Vesicle-Mycorrhizae, manufactured by: JH Biotech, INC, Ventura USA; imported and distributed in Australia by Zadco for Quality Gro PTY LTD); this was applied at 4 g/L as per label recommendation, and mixed into the soil surface without disturbing the

seedling roots. The components of MYCORMAX product are described in Table 5.3. After transplanting, plants were grown for more than 11 weeks (82 days) under natural lighting conditions. Glasshouse temperature was 20±2° C. To control whiteflies, the glasshouse was fumigated by Pestigas (0.4% natural pyrethrums, 2% Piperonyl Butoxide in Carbon) at week 8.

Table 5.2 The compositional formula for organic applications used in the trial.

	N (mg/kg)	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Cl (mg/kg)
Compost	15000	7500	6100	2600	13100	9800	1800	18100	3700
Ferbon	13400	1990	4620	17700	14800	2790	2460	9100	NA

	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	B (mg/kg)	Mo (mg/kg)	PH	Electrical Cond.
Compost	383.3	199.9	65.1	6.7	30.8	4.4	6.5	2000 uS/cm
Ferbon	488.0	142.0	91.0	11.8	54.7	9.0	6.2	3255 uS/cm
Ferbon	Org. C 37.5 % and Moisture Con. 35.4 %							

Table 5.3 The compositional formula for the AMF application used in the trial.

Treatment	Constituents
AMF (Mycormax™, 2010)	Active Ingredients 2%, Inert Ingredients 98% Glo*mus intraradices (Rhizophagous irregularis)* 46 spores/gm, *Glomus mosseae* 19 CFU/gm. ectomycorrhizal fungi*: *Laccaria bicolor* 500 CFU/gm, *Pisolithus tinctorius* 15,300 CFU/gm, *Scleroderma cepa* 1,760 CFU/gm, *Scleroderma geastrum* 1,760 CFU/gm and *Scleroderma citrinum* 1,760 CFU/gm.

**Note that the ectomycorrhizal fungi Pisolithus tinctorius, Scleroderma cepa, Scleroderma geastrum, Scleroderma citrinum* and *Laccaria biocolorare would not have colonized sunflower. The asterids class (including the Asteraceae order, which is where sunflower is classified) are dominated by AM and ericoid mycorrhizal partnerships (Brundrett, 2009).*

5.3.3 Assessments

5.3.3.1 Canopy

Leaf chlorophyll content was assessed with a Minolta SPAD-502 meter at 4, 5, 6, 7, 8 and 9 weeks. Four fully expanded mature leaves were selected on each plant at each assessment date. Plant height and number of nodes were assessed at 5, 6, 7, 8 and 9 weeks. Stem diameter was measured at the fourth internode. Diameter of the primary flower head and number of axillary flowers was measured in week 10. Flowering dates were recorded daily from the beginning of flowering until the last plant flowered. After flowering the stem was cut at the soil surface (week 12), and plants weighed to determine fresh weight. Plant stems

(with leaves and flower heads) were dried at 40° C, until weight was stable and then dry weight was measured.

Dry matter content was defined as: Dry weight = $\frac{\text{dry weight}}{\text{fresh weight}}\ x\ 100$

5.3.3.2 Nutrient analysis

After drying at 40° C leaves were taken from each plant from node 5 upwards and ground with a coffee grinder to a fine powder prior to bagging. Roots were removed from each pot following removal of the stems and the soil was allowed to air dry. Once dry, soil from each pot was sub-sampled and samples placed into labelled plastic bags. Leaf and soil samples were sent to a commercial laboratory for analysis (Soil & Plant Laboratory, CSBP Limited, Western Australia).

5.3.3.3 Root colonisation of AMF

To estimate mycorrhizal colonisation in sunflower roots at the end of the trial, roots were gently shaken to remove soil, washed and taken to the laboratory. Fine roots branching from coarse roots were selected, washed with tap water and cut into 1–1.5 cm segments. Roots were cleaned by heating for 3-5 minutes in 5% KOH. The roots were then strained and rinsed twice with tap water, and again rinsed in 3.5% HCl. Roots were stained with 5% black Sheaffer ink in lactic acid according to methods described by Khaosaad *et al.* (2006) and Toussaint *et al.* (2007) by reheating for 3 minutes. Roots were rinsed once with tap water and placed in water with a few drops of lactic acid to destain.

5.3.3.4 Estimating AMF colonisation

Following the method described by McGonigle *et al.* (1990), five slides (five roots for each slide) of stained roots were prepared; roots were placed horizontally, mounted in water, and covered by coverslips. Using a crosshair eyepiece 30 root intersects per slide were inspected, scoring for presence/absence of hyphae, arbuscules and vesicles.

Hypha (H), vesicle (V) or arbuscule (A) presence = $\frac{\text{X}}{150}x100$

Where (X) = hypha, vesicle or arbuscule

5.3.4 Statistical analysis

Data was analysed by analysis of variance using RStudio (Version 0.99.473 – © 2009-2015 RStudio, Inc.). Data of AMF colonisation were transformed into LOG (x + 1) only when necessary for normality distribution. The data is displayed as mean values for each treatment.

Significance between treatments was tested at $P < 0.05$ and least significant difference (LSD) used to compare treatment means. Sigma Plot v 12.5 was used to draw graphs.

5.4 Results

5.4.1 Mycorrhizal Colonisation

The roots of sunflower plants that were treated with MYCORMAX were colonised by arbuscule vesicle-mycorrhizae, although colonisation by AMF also occurred in plants that were not inoculated (Figure 5.1 and 5.2).

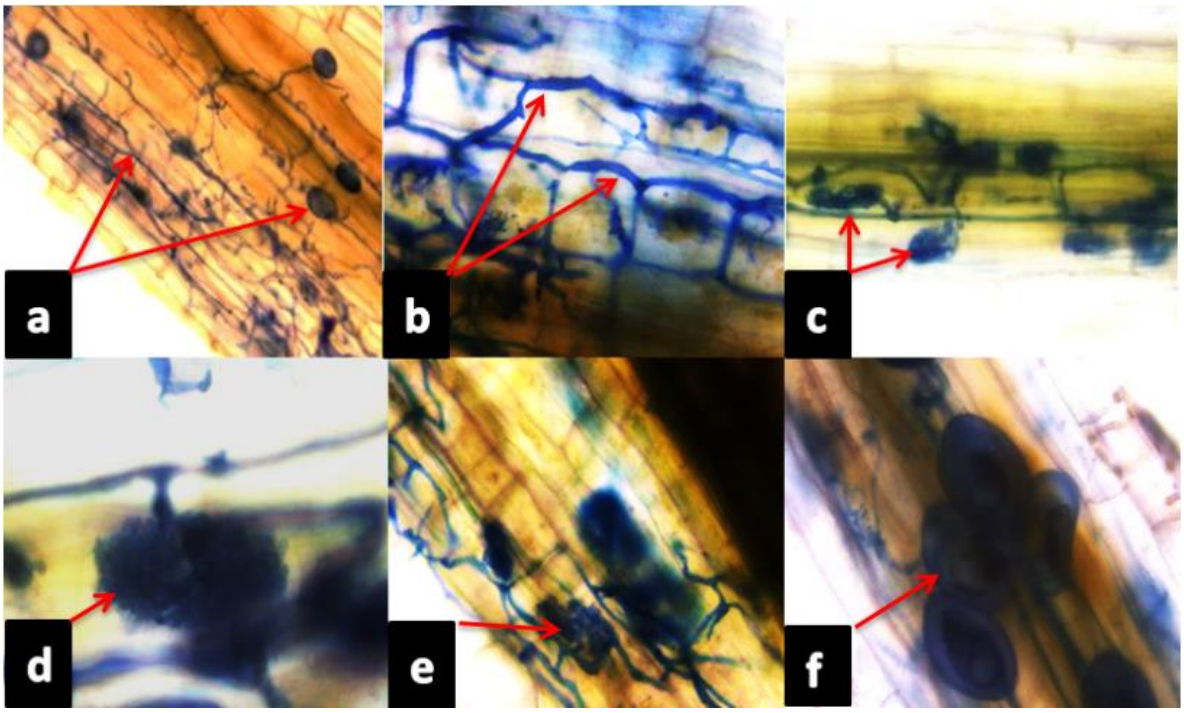


Figure 5.1 (a) Stained vesicles and hypha in roots of compost treated plants under 200x lenses (forest soil). (b) Stained hyphae in roots of AMF treatment under 1000x lens (orchard soil). (c) Stained arbuscules within a cleared cell of sunflower root (Ferbon treatment in forest soil). (d) Stained arbuscules for Ferbon treatment in orchard soil (lens 400x). (e) stained arbuscules of mycorrhizal fungi in roots of compost + SHG treatment. (f) Stained vesicles for treatment of SHG in forest soil (light microscope lens 400X).

Interactions were found between AMF inoculation and organic supplements for hyphal and arbuscule colonisations in the orchard soil only. Addition of AMF to compost, compost plus SHG and control treatments increased hyphal colonisation in the orchard soil compared to treatments where AMF was not added (Figure 5.2a). There was a significant interaction between AMF and organic supplements for arbuscule colonisation (Figure 5.2b). There were no interaction effects between treatments on mycorrhizal colonisation in the forest soil (data not shown).

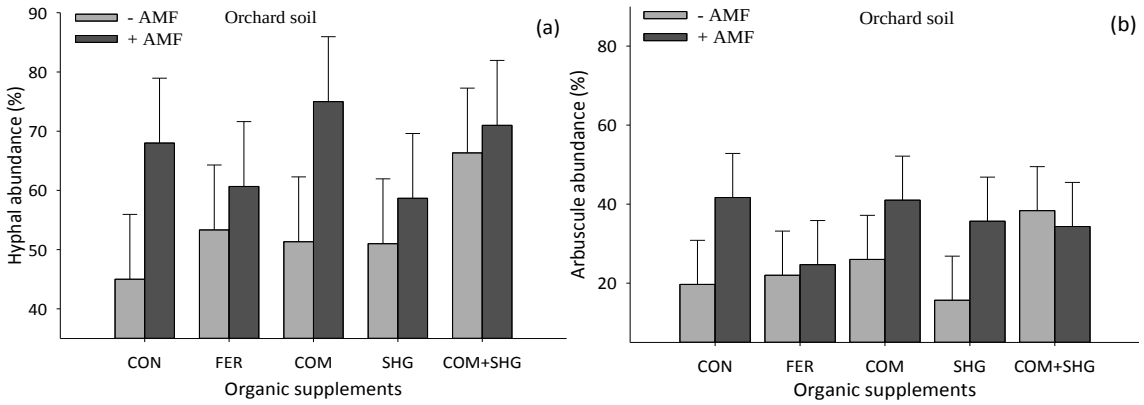


Figure 5.2 Interaction effect between the inoculation of arbuscule mycorrhizal fungi (AMF) and organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)) on hyphal and arbuscule colonisations in orchard soil. Error bars represent LSD values.

Mycorrhizal application significantly increased the presence of hyphae and arbuscules in forest soil (Table 5.4) and all colonisation structures increased in the orchard soil (Table 5.5). In the forest soil, significant effects on all colonisation structures were found with Ferbon (Table 5.4), but the effect did not take the same pattern in the orchard soil (Table 5.5). Plants with applied compost showed a significant increase in presence of hyphae and arbuscules in the forest soil, whereas SHG had significant effect on hyphal presence in the forest soil only, but there were no differences between compost + SHG and Control (Table 5.4). In the orchard soil, compost increased the presence of hyphae and arbuscules but adding SHG had no additional effect (Table 5.5).

Table 5.4 Treatment main effects on arbuscule vesicle-mycorrhizae (AMF) colonisation in sunflower roots in forest soil. SHG = soluble humate granules and COM = compost.

	Hypha (%)	Vesicles (%)	Vesicles LOG (x +1)	Arbuscules (%)	Arbuscules LOG (x +1)
			(i) AMF inoculation		
- AMF	41.5 b	12.8	1.11	25.9 b	1.40 b
+ AMF	49.5 a	15.9	1.17	34.0 a	1.54 a
L.S.D.	*6.2*	*ns*	*ns*	*5.1*	*0.09*
F. Prop.	*0.01*	*0.08*	*0.28*	*0.003*	*0.005*
			(ii) Organic supplements		
Control	37.8 b	10.5 b	0.98 c	23.7 c	1.37 c
Ferbon	54.7 a	23.8 a	1.38 a	34.2 ab	1.53 ab
Compost	51.7 a	14.8 b	1.20 b	36.7 a	1.57 a
SHG	46.2 ab	13.7 b	1.13 bc	27.3 bc	1.42 bc
COM + SHG	37.2 b	9.3 b	1.01 c	27.8 bc	1.45 abc
L.S.D.	*9.80*	*5.60*	*0.18*	*7.90*	*0.14*
F Prop.	*0.003*	*<0.001*	*0.001*	*0.02*	*0.04*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 5.5 Treatment main effects on arbuscule vesicle-mycorrhizae (AMF) colonisation in sunflower roots in orchard soil. SHG = soluble humate granules and COM = compost.

	Hypha (%)	Vesicles (%)	Arbuscules (%)
		(i) AMF inoculation	
- AMF	53.4 b	23.7 b	24.3 b
+ AMF	66.7 a	28.2 a	35.5 a
L.S.D.	*4.80*	*3.80*	*4.90*
F. Prop.	*<0.001*	*0.02*	*<0.001*
		(ii) Organic supplements	
Control	56.5 bc	25.5	30.7 abc
Ferbon	57.0 bc	26.0	23.3 c
Compost	63.2 ab	26.2	33.5 ab
SHG	54.8 c	22.7	25.7 bc
COM + SHG	68.7 a	29.3	36.3 a
L.S.D.	*7.70*	*ns*	*7.80*
F Prop.	*0.04*	*0.30*	*0.01*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

5.4.2 Growth properties

Although not many significant differences occurred on different growth characteristics, there also were no negative effects. Organic supplements along with inoculation by AMF improved some growth characteristics. There was a significant interaction between organic supplements for leaf chlorophyll content. The interaction between organic supplements and AMF inoculation had significant effects on leaf chlorophyll content at week 7 (P = 0.02) and week 8 (P = 0.03) in the orchard soil, but the effects were not consistent between treatments (data not shown). There were no significant differences found for either AMF or organic supplements on leaf chlorophyll content, flower number, flower time and dry weight percentage (data not shown). Mycorrhizal inoculation also had no effect on plant height in both soils (data not presented), but significant differences were found with organic supplements in forest soil on plant height. Compost increased plant height from week 7 (P = 0.02), week 8 (P = 0.007), week 9 (P = 0.002) and week 10 (P = 0.004), but there were no significant differences between the compost and control treatments (Figure 5.3).

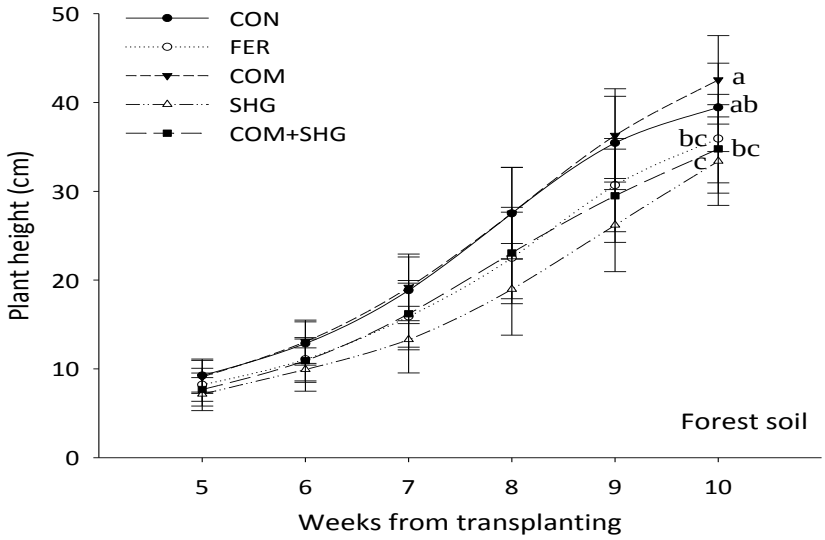


Figure 5.3 Main effect of organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)) on plant height for sunflowers grown in forest soil. Error bars represent LSD values.

There were no interactions between AMF and organic supplements for number of nodes, dry weight, flower diameter, flower number and time of flowering. Stem diameter was reduced by both SHG - AMF and compost + SHG + AMF treatments (Figure 5.4a). The number of nodes for treatments with AMF added was significantly increased at week 5 (P = 0.03) and week 6 (P = 0.04) in the forest soil, but the effect did not carry through to the end of the trial. In the orchard soil, the number of nodes was not affected by treatments (data not shown).

Soluble humate granules increased stem diameter (P = 0.03) in forest soil compared with other organic supplements, but no differences were found between SHG and control treatments (Figure 5.4b).

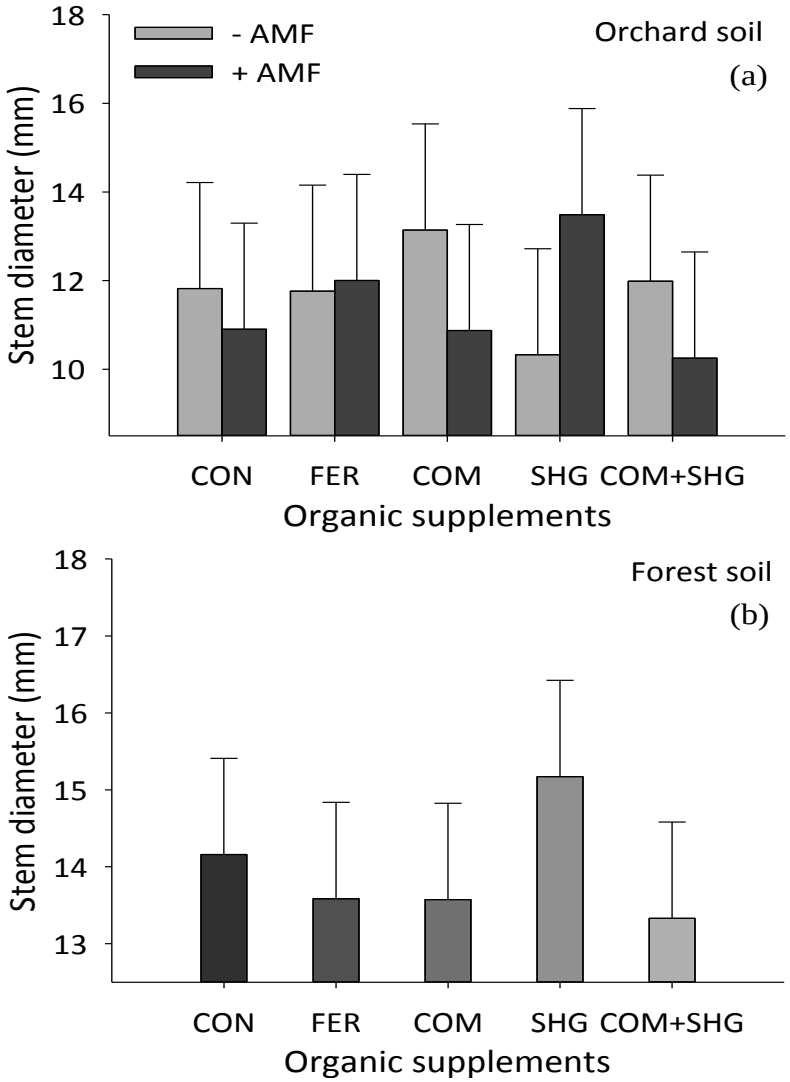


Figure 5.4 Effects of treatments on stem diameter of pot-grown sunflower. a) interaction between organic supplements and mycorrhizal inoculation (AMF) on stem diameter, b) Main effect of organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)). Error bars represent LSD values.

Compared with the control, flower diameter increased up to 22% and 24% with Ferbon and SHG respectively in orchard soil (P < 0.001), but the same treatments reduced flower diameter in the forest soil (P = 0.03) (Figure 5.5a). Mycorrhizal inoculation significantly increased the primary flower head diameter in orchard soil at P = 0.04 (Figure 5.5b), but had no effect on dry weight, flower diameter, number and time (data not shown).

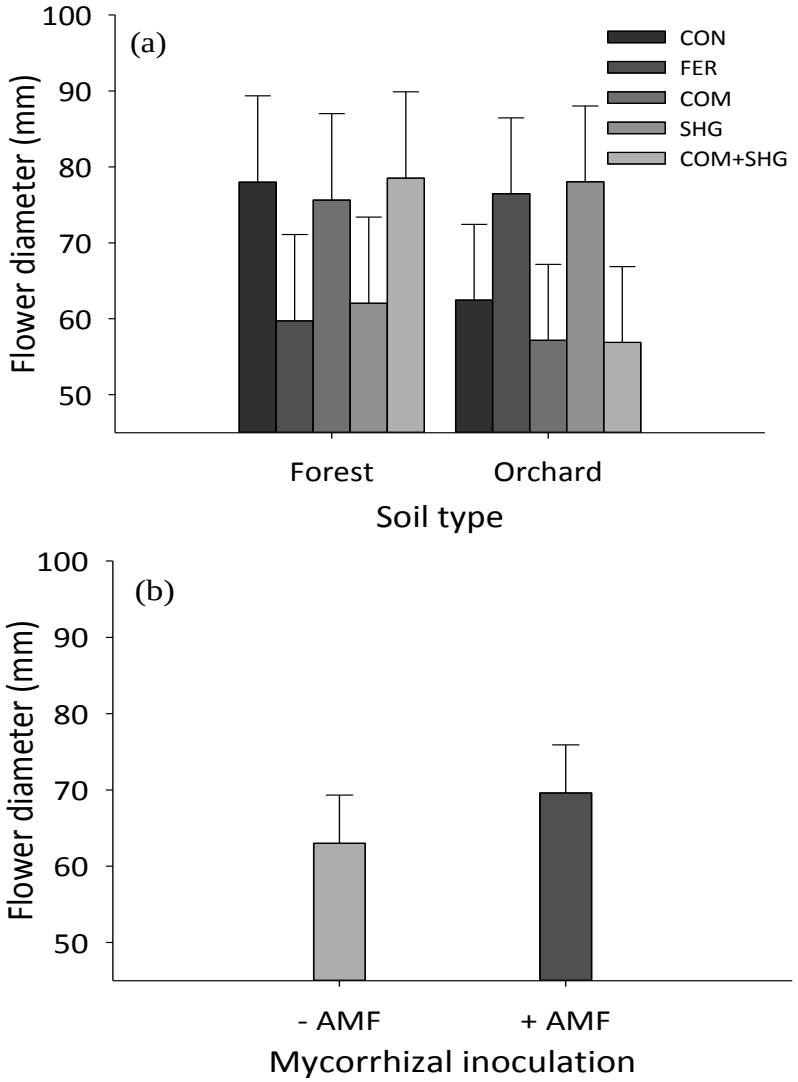


Figure 5.5 a) Main effect of organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)), and b) mycorrhizal colonisation on flower head diameter in two types of soil. Error bars represent LSD values.

5.4.3 Leaf nutrient status

The organic supplements all showed a similar effect on foliar nutrient concentrations except for total foliar N. A significant interaction (P = 0.03) was found between AMF and organic supplements for total foliar N in plants grown in the forest soil, but there was no outstanding treatment (Figure 5.6). There were no significant interactions for other foliar nutrients. In the orchard soil, the combined compost + SHG treatment minus AMF resulted in higher leaf nutrient levels for Ca (P = 0.04), Mn (P = 0.01) and Zn (P = 0.04) (data not shown).

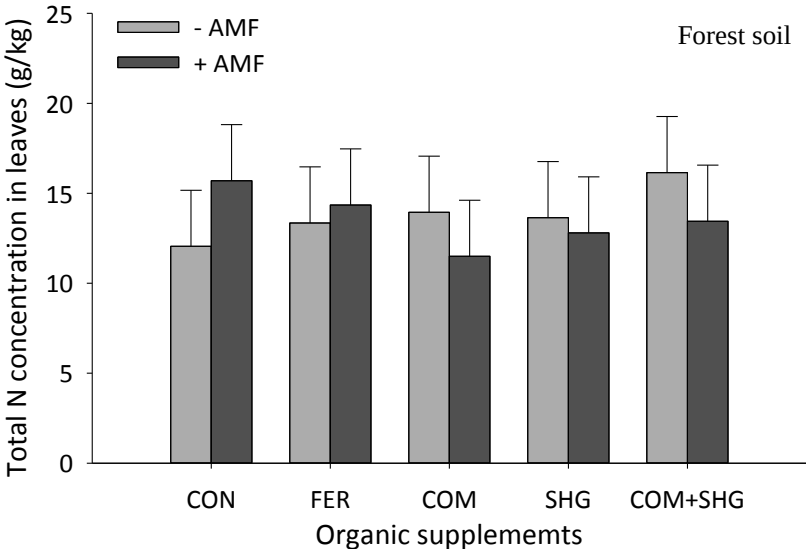


Figure 5.6 Interaction effect between organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)) and the inoculation of arbuscule mycorrhizal fungi (AMF) on total nitrogen in sunflower leaves. Error bars represent LSD values.

In the forest soil, mycorrhizal inoculation significantly increased foliar K, decreased foliar Mg, reduction in Zn is unexpected and had no effect on other leaf nutrients (Table 5.6). Foliar nutrient levels were not affected by additions of organic supplements (Table 5.6).

Table 5.6 Main effect of treatments on concentrations of nutrients at 12 weeks in sunflower leaves of plants grown in forest soil. AMF = arbuscule mycorrhizal fungi, SHG = soluble humate granules and COM = compost.

	Total N (g/kg)	P (g/kg)	K (g/kg)	Mg (g/kg)	Ca (g/kg)	B (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
(i) AMF inoculation									
- AMF	13.8	1.5	15.9 b	13.8 a	39.9	110	421	58	158 a
+ AMF	13.6	1.6	21.1 a	9.9 b	39.4	129	370	96	118 b
L.S.D.	*ns*	*ns*	*4.55*	*1.68*	*ns*	*ns*	*ns*	*ns*	*24*
F. Prob.	*0.65*	*0.13*	*0.03*	*<0.001*	*0.86*	*0.12*	*0.18*	*0.07*	*0.005*
(ii) Organic supplements									
Control	13.9	1.5	19.8	12.4	42.8	117	435	73	159
Ferbon	13.9	1.4	18.1	12.5	41.5	133	431	69	147
Compost	12.7	1.4	15.3	13.1	40.7	111	391	68	153
SHG	13.2	1.7	19.8	10.5	37.8	132	359	116	112
COM + SHG	14.8	1.8	19.5	10.8	35.4	105	361	60	119
L.S.D.	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
F Prob.	*0.30*	*0.12*	*0.59*	*0.19*	*0.45*	*0.46*	*0.53*	*0.39*	*0.07*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

In the orchard soil, mycorrhizal inoculation increased foliar K and reduced foliar Ca but had no effect on other leaf nutrients (Table 5.7). Compared with the control, compost + SHG and Ferbon treatments increased leaf K, while Ferbon increased leaf Zn and Mn.

Table 5.7 Main effect of treatments on concentrations of nutrients at 12 weeks in sunflower leaves of plants grown in orchard soil. AMF = arbuscule mycorrhizal fungi, SHG = soluble humate granules and COM = compost.

	Total N (g/kg)	P (g/kg)	K (g/kg)	Mg (g/kg)	Ca (g/kg)	B (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
				(i) AMF inoculation					
- AMF	15.4	2.2	15.7 b	12.1	27.3 a	76	496	697	125
+ AMF	16.5	2.2	20.4 a	12.0	23.9 b	75	454	613	107
L.S.D.	*ns*	*ns*	*3.32*	*ns*	*2.53*	*ns*	*ns*	*ns*	*ns*
F. Prob.	*0.32*	*1.00*	*0.01*	*0.92*	*0.01*	*0.9*	*0.18*	*0.49*	*0.17*
				(ii) Organic supplements					
Control	14.1	2.0	14.8 b	11.5	24.2	61 b	407 b	566	106 bc
Ferbon	16.0	2.1	19.2 ab	13.2	28.8	104 a	561 a	729	150 a
Compost	14.8	2.4	17.1 b	12.4	25.6	77 b	494 ab	624	100 bc
SHG	16.0	2.1	15.8 b	11.5	23.5	61 b	410 b	684	90 c
COM + SHG	18.8	2.2	23.4 a	12.0	25.8	73 b	502 ab	674	134 ab
L.S.D.	*ns*	*ns*	*5.25*	*ns*	*ns*	*21*	*102*	*ns*	*41*
F Prob.	*0.08*	*0.55*	*0.03*	*0.51*	*0.10*	*0.007*	*0.03*	*0.92*	*0.04*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

5.4.4 Soil nutrient status

The forest soil showed initial higher levels of most nutrients as well as organic C than the orchard soil (Table 5.1). However, the addition of organic supplements in conjunction with inoculation by AMF led to significant effects on nutrient levels within each soil. Significant interactions were found with AMF plus organic supplements on soil pH, the level of NH_4^+-N, NO_3^- - N, P, K, Exc. K, Exc. Ca, Exc. Mg, B, Mn, Fe and Zn in the forest soil; and soil pH, P, Exc. Ca, Exc. Mg, Mn, Fe and Zn in the orchard soil (data not shown). Compared with the control, soil P levels in the orchard soil were similar for all organic supplement treatments (Figure 5.7a). AMF inoculation increased P levels in the control, compost and compost + SHG treatments compared to the respective non-inoculated treatments. Zinc levels were increased in the Ferbon, compost and compost + SHG, but not the SHG treatment. AMF inoculation increased Zn in Ferbon, and SHG treatments, but reduced Zn in the compost + SHG treatment (Figure 5.7b).

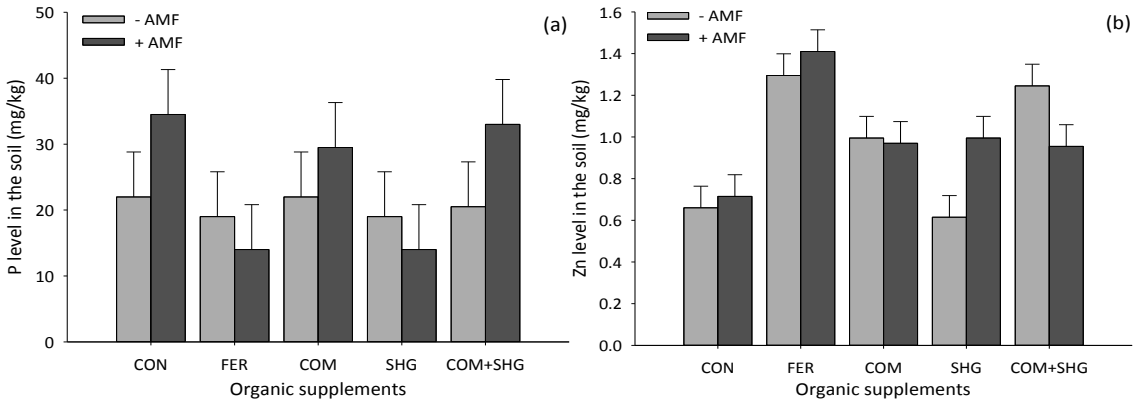


Figure 5.7 Interaction effect between organic supplements (control (CON), Ferbon (FER), compost (COM) and soluble humate granules (SHG)) and the inoculation of arbuscule mycorrhizal fungi (AMF) on a) phosphorus level and b) zinc level in the orchard soil post-harvest. Error bars represent LSD values.

Forest soil: levels of NH_4^+-N, NO_3^--N, P, K, Mn and Zn were lower in AMF inoculated soils compared with non-inoculated (Table 5.8), while exchangeable Ca (Exc. Ca) increased (Table 5.9). Concentrations of K, Exc. K, Exc. Mg, Exc. Ca, B and Mn were higher in soils amended with Ferbon, but there were no differences between Ferbon and control treatments for Exc. Mg concentrations (Tables 5.8 and 5.9). Soils amended with SHG had higher levels of NH_4^+-N, NO_3^--N, P, K, Exc. K and B compared with either compost or compost + SHG (Tables 5.8 and 5.9). Levels of Mn, Fe and Zn were higher in soils treated with organic supplements compared with control soil (Table 5.9). Soil pH increased in soils inoculated by AMF but decreased in soils treated with SHG (Table 5.9).

Table 5.8 Main effect of treatments on soil nutrient status at 12 weeks in the forest soil. AMF = arbuscule mycorrhizal fungi, COM = compost and SHG = soluble humate granules.

	NH_4^+ - N (mg/kg)	NO_3^- - N (mg/kg)	P (mg/kg)	K (mg/kg)	Exc. K (meq/100g)	Exc. Mg (meq/100g)
(i) AMF inoculation						
- AMF	118.9 a	47.2 a	137.7 a	547 a	1.28	7.8
+ AMF	74.1 b	24.3 b	97.1 b	464 b	1.19	7.8
L.S.D.	*9.91*	*3.32*	*18.89*	*61.85*	*ns*	*ns*
F. Prob.	*<0.001*	*<0.001*	*<0.001*	*0.01*	*0.17*	*0.13*
(ii) Organic supplements						
Control	98.0 b	28.0 d	144.3 a	436 b	1.14 b	8.3 a
Ferbon	92.0 b	41.0 b	102.5 bc	622 a	1.53 a	8.3 a
Compost	55.5 c	20.3 e	75.8 c	350 b	0.90 c	7.7 b
SHG	151.8 a	54.5 a	155.8 a	702 a	1.56 a	7.7 b
COM + SHG	85.3 b	35.0 c	108.8 b	418 b	1.05 bc	7.4 c
L.S.D.	*15.68*	*5.26*	*29.87*	*97.79*	*0.23*	*0.19*
F Prob.	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 5.9 Main effect of treatments on soil nutrient status at 12 weeks in the forest soil. AMF = arbuscule mycorrhizal fungi, COM = compost and SHG = soluble humate granules.

	Exc. Ca (meq/100g)	B (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Soil pH
(i) AMF inoculation						
- AMF	22.4 b	0.88	101 a	242	25.2 a	5.22 b
+ AMF	23.1 a	0.88	90 b	240	23.4 b	5.39 a
L.S.D.	*0.29*	*ns*	*3.89*	*ns*	*0.67*	*0.03*
F. Prob.	*<0.001*	*0.95*	*<0.001*	*0.39*	*<0.001*	*<0.001*
(ii) Organic supplements						
Control	22.9 b	0.78 c	88 b	223 c	23.6 b	5.38 a
Ferbon	24.1 a	0.99 a	100 a	235 b	23.7 b	5.38 a
Compost	22.2 c	0.70 d	97 a	259 a	25.6 a	5.30 b
SHG	21.9 c	1.03 a	96 a	256 a	24.6 ab	5.15 c
COM + SHG	22.7 b	0.89 b	96.12 a	229 bc	23.9 b	5.33 ab
L.S.D.	*0.47*	*0.06*	*6.15*	*8.70*	*1.05*	*0.06*
F Prob.	*<0.001*	*<0.001*	*0.01*	*<0.001*	*0.007*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Orchard soil: Levels of NH_4^+-N, NO_3^--N, P, K, Exc. K, Exc. Mg, Exc. Ca and Zn were higher in AMF inoculated soils compared with non-inoculated (Tables 5.10 and 5.11), while

Fe level was lower (Table 5.11). Compared with the control, all organic supplements increased Exc. Mg, Exc. Ca, Mg, Mn and Fe in the soil with compost having the greatest effect, while soils treated with SHG had lower NH_4^+-N, NO_3^--N, K, and Exc. K compared to other organic supplements (Tables 5.10 and 5.11). Additions of Ferbon and SHG decreased P level in the soil compared with other treatments including control (Table 5.10). Boron level was not affected by the additions of organic supplements (Table 5.11). Soil pH increased in soils inoculated by AMF and in soils treated with compost and SHG but SHG had no additional effect compared with compost or SHG alone (Table 5.11).

Table 5.10 Main effect of treatments on soil nutrient status at 12 weeks in orchard soil AT. AMF = arbuscule mycorrhizal fungi, SHG = soluble humate granules and COM = compost.

	NH_4^+ - N (mg/kg)	NO_3^- - N (mg/kg)	P (mg/kg)	K (mg/kg)	Exc. K (meq/100g)	Exc. Mg (meq/100g)
(i) AMF inoculation						
- AMF	7.4 b	3.5 b	20.5 b	44 b	0.11 b	0.16 b
+ AMF	10.7 a	4.1 a	25.0 a	59 a	0.15 a	0.18 a
L.S.D.	*2.28*	*0.54*	*3.04*	*6.75*	*0.01*	*0.008*
F. Prob.	*0.009*	*0.03*	*0.008*	*<0.001*	*<0.001*	*<0.001*
(ii) Organic supplements						
Control	14.5 a	4.3 ab	28.3 a	58 ab	0.15 ab	0.13 d
Ferbon	7.8 bc	4.0 ab	16.5 b	49 b	0.13 b	0.15 c
Compost	8.8 b	3.8 b	25.8 a	60 a	0.16 a	0.23 a
SHG	5.0 c	2.3 c	16.5 b	38 c	0.09 c	0.16 c
COM + SHG	9.3 b	4.8 a	26.8 a	54 ab	0.14 ab	0.18 b
L.S.D.	*3.61*	*0.86*	*4.81*	*10.67*	*0.02*	*0.01*
F Prob.	*0.002*	*<0.001*	*<0.001*	*0.006*	*0.002*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 5.11 Main effect of treatments on soil nutrient status at 12 weeks in orchard soil AT. AMF = arbuscule mycorrhizal fungi, SHG = soluble humate granules and COM = compost.

	Exc. Ca (meq/100g)	B (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Soil pH
			(i) AMF inoculation			
- AMF	0.66 b	0.16	1.4	50 a	0.96 b	5.58 a
+ AMF	0.70 a	0.17	1.5	43 b	1.01 a	5.48 b
L.S.D.	*0.02*	*ns*	*ns*	*1.48*	*0.04*	*0.099*
F. Prob.	*0.005*	*0.49*	*0.48*	*<0.001*	*0.04*	*0.04*
			(ii) Organic supplements			
Control	0.52 e	0.16	1.1 d	39 c	0.69 e	5.38 c
Ferbon	0.65 d	0.18	1.5 bc	44 b	1.35 a	5.40 bc
Compost	0.80 a	0.19	1.8 a	54 a	0.98 c	5.55 ab
SHG	0.69 c	0.17	1.4 c	43 b	0.81 d	5.65 a
COM + SHG	0.74 b	0.17	1.6 b	52 a	1.10 b	5.68 a
L.S.D.	*0.04*	*ns*	*0.14*	*2.35*	*0.07*	*0.15*
F Prob.	*<0.001*	*0.20*	*<0.001*	*<0.001*	*<0.001*	*0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

5.5 Discussion

Results of this trial have highlighted that the impact of amendments (both AMF and organic supplements that contain humic substances) is greater in low nutrient soil, as demonstrated in the comparison between the forest soil and the depleted orchard soil.

5.5.1 Soil fertility level and AMF performance

The lack of effect of AMF treatment on plant growth attributes in response to different levels of soil nutrient could be a result of the inability of the AMF species [Glo*mus intraradices (Rhizophagous irregularis)* and *Glomus mosseae*] used in this trial to uptake nutrients for this host plant and hence change the nutritional status. Demir *et al.* (2015) reported that the species of AMF play an important role in macro and micro-nutrient uptake with low concentrations of P in the soil. Additionally, a change in plant growth depends on the change in the status of nutrients within the plant tissue. Forde (2002) reported that plants show a high degree of physiological flexibility and growth response to changing nutritional circumstances.

The convergence of the growth of mycorrhizal plants may not be only due to the performance of the AMF species used, but could also be due to increase in the supply of nutrients, especially essential nutrients such as NPK. Therefore, plants may have obtained sufficient

nutrients already present in the soil, which in turn minimised the role played by mycorrhizae. Mäder *et al.* (2000) reported that in high-nutrient nutrition conditions, plants stop providing their fungal partners with sources they need. This in turn leads to a reduction of colonisation, and the role of symbiotic associations between AMF and the host plant becomes less important.

5.5.2 Additions of organic supplements and AMF performance in the soil

The results indicate that the release of available nutrients such as N, Ca, Mn and Zn in the soil can improve by adding organic supplements with AMF inoculation. The type of organic supplement may be the main reason in determining the rate at which nutrients are mineralised. In this study, the organic supplements differed in their influence on both nutrient availability in the soil and the degree of AMF colonisation. The organic supplements may have caused changes in soil chemical and physical properties such as pH and water retention. These factors in turn affect both nutrient availability and AMF colonisation (low or high P released). The increase in AMF colonisation may lead to an increase in organic compounds exuded by plant roots in the soil, which also apparently contribute to the mineralisation of nutrients in the soil. Tadano and Sakai (1991) mentioned that roots of mycorrhizal plant species secrete a larger amount of organic compounds including amino acids and carbohydrates compared to roots of non-mycorrhizal plant species. Organic acids can modify the rhizosphere pH and consequently, the potential change in soil pH caused by the additions of organic supplements and/or the increase the AMF colonisation may be the reason behind the increased availability of nutrients in the soil. According to Lyle *et al.* (2006) soil acidity plays a major role in the availability of nutrients in the soil.

5.5.3 Humic material additives and nutrient availability

Even though similar growth patterns were noticed in most growth attributes of organic supplemented plants, the humic supplements (Ferbon and SHG) were found to have facilitated greater release of nutrients in the soil compared to compost. Addition of SHG to the compost increased the availability of most nutrients in the less fertile orchard soil, improving plant growth. In contrast, the compost performance was better when added to the forest soil. The availability of many mineral nutrients in the soil is significantly affected by soil pH (Lyle *et al.,* 2006). Compost and SHG together reduced soil pH from 5.7 to 4.6 in the forest soil. This may be due to other factors such as C:N or C:P ratio which were not considered in this trial. It is possible that the soil pH may have played an important role in the

orchard soil, resulting in the decrease in soil pH from 5.9 to 5.6 and 5.9 to 5.5 respectively with SHG and compost.

According to Plaza *et al.* (2006) and Xiong *et al.* (2010), the significant influence of humic substances in facilitating mineral nutrient release, especially heavy mineral nutrients such as zinc, is due to the high molecular weight of these humic substances with low acidic functional groups. Plaza *et al.* (2006) state that because humic substances have a smaller molecular weight and contain larger acidic functional groups they can increase or decrease the liberation of nutrients in the soil. This occurs through the formation of complex compounds, either soluble or insoluble, with metal ions, depending on the soil pH value in the soil. Consequently, humic substances can play a dual role in the soil. In the current trials, the orchard soil pH may be had at the appropriate level for the formation of soluble complex compounds with humic substances, while the acidity of the forest soil may be enhanced of the formation of insoluble complex compounds, at least with some nutrients.

Soil pH plays a major role in facilitation and absorption of nutrients in the soil (Hyland *et al.*, 2005, Lyle *et al.*, 2006). Most organic supplements increased orchard soil pH but reduced forest soil pH in this study. This increase in soil pH resulting from addition of organic supplements in the orchard soil is likely to have improved nutrient availability.

5.5.4 Additions of organic supplements and mycorrhizal colonisation

While compost and Ferbon additions had a major influence on AMF colonisation in this study, the effect appears to be dependent on soil type. The observed increase in AMF colonisation could be due to a decrease in the level of mineralised-P into the forest soil, as a result of plant uptake and leaching through irrigation water. Compost and Ferbon treatments contained lower P compared to the control treatment, which may have led to increase AMF colonisation in these treatments in the forest soil. This is in agreement with Cavagnaro (2014) who reported that root colonisation by AMF decreased from 85% to 60% and 40% when P-concentrations increased in the soil from 4 up to 20 and 76 mg/kg available P in dry soil. Furthermore, Baon *et al.* (1992) observed that the release of larger quantities of mineralised P into the soil decreased mycorrhizal colonisation.

In relation to the orchard soil, low level of P in the compost and SHG treatments may have had a positive impact on AMF colonisation, where the level of mineralised-P was not too high to prevent AMF colonisation. Clearly, the level of mineralised-P in the soil solution was at the required level to stimulate root colonisation by AMF. Plant response to colonisation

may differ between different soils depending on the availability of other nutrients such as Zn, as observed with compost and SHG supplements. According to Zhu *et al.* (2001), increase in Zn concentration in the soil resulted in an increase in mycorrhizal colonisation in white clover roots.

The above explanations may not be applicable to all types of soils. The role of organic supplements in promoting AMF colonisation and plant growth may differ depending on the soil type. Although P content in SHG and compost treatments was higher than the other treatments, AMF colonisation was at its peak in these treatments in the orchard soil. Compost normally contains propagules of AMF and this may be responsible for the increase in AMF colonisation in the orchard soil as observed by Cavagnaro (2015) and Fernandes *et al.* (1994). Furthermore, the application of compost to the orchard soil may have added other species of AMF, thus increasing AMF colonisation. Higher nutrient content in the forest soil may have been the cause of the lack of effect of AMF colonisation observed with addition of compost.

5.6 Conclusion

The results of this study showed that the improvement of sunflower growth is possible with commercial AMF inoculation under both low and high-nutrient soils. In the presence of a sufficient level of nutrients in the soil, AMF inoculation can enhance some foliar nutrients including N and K. Nutrient availability in both soil types was strongly influenced by the organic supplements, especially humic supplements. In comparison with compost, humic supplements combined with AMF inoculation may not directly impact on plant productivity under excessive presence of nutrients; however they can enhance AMF colonisation, the status of foliar nutrients and the health and fertility of the soil by regulating the release of nutrients. The nutritional requirements of different crops may also affect the impact of AMF inoculation and organic supplements on plant growth.

"Chapter 6" Changes in arbuscular mycorrhizal colonisation with different nutrient and orchard floor management practices and relationship to flavour of apple and cherry fruit

6.1 Abstract

The degree of colonisation structures (hyphae, vesicles and arbuscules) of arbuscular mycorrhizal fungi (AMF) in apple (*Malus domestica* Borkh and sweet cherry (*Prunus avium* L. cultivar 'Lapin' and 'Sweetheart') roots was assessed for three seasons across three established orchards under different nutrient regimes. The degree of AMF colonisation and the relationship to the development of flavour characteristics (total soluble solids (TSS), titratable acidity (TA) as malic acid, and the ratio of TSS/TA for apple and cherry fruit were evaluated. In apple roots, nutrient applications had no effect on all AMF colonisation structures under all assessment seasons. Compared to herbicide and clover/grass orchard floor treatments, biochar additions significantly increased all colonisation structures as well as total AMF colonisation in all seasons, except total AMF colonisation in autumn 2014. In cherry roots significant interactions between nutrient regime and effective microbes (EM) inoculation were detected for arbuscule, vesicle and total AMF colonisation. The presence of arbuscules in spring 2014 was significantly reduced in the conventional plus EM treatment in roots of 'Lapin'; but decreased in 'Sweetheart' roots with the conventional minus EM treatment compared with other treatment combinations. Vesicle abundance and AMF colonisation during summer 2014 significantly increased with conventional regime minus EM in 'Lapin', while the effect was inconsistent in 'Sweetheart'. This means that there was no clear indication as to which of the treatments was the driving factor when no interaction effects were noted. Furthermore, EM inoculation significantly decreased the formation both of vesicles and arbuscules in 'Lapin' (spring 2013) but had no effect in subsequent seasons. Additionally, it did not have any effect on the formation of vesicles in the spring of 2013 in 'Sweetheart', but it increased the formation of arbuscules in spring 2013 and 2014. In apple 'the degree of AMF colonisation correlated positively both to fruit TSS content and TA level, but negatively to the TSS/TA ratio. There was also a strong positive correlation between TSS content and TA level and type of nutrient regime, specifically, between TSS content and the alternative regime, and TA level and conventional nutrient regime. TSS content also correlated positively to herbicide and clover/grass treatments but not to biochar, while TSS/TA ratio negatively correlated to total AMF colonisation and both conventional and clover/grass treatments. In cherry fruits, TSS content positively correlated to overall AMF

colonisation and conventional treatment in both cultivars. TA level strongly correlated to conventional treatment in 'Sweetheart' cultivar, while in 'Lapin', TSS/TA ratio positively correlated with overall AMF colonisation. Overall, the results of the study confirmed that, while the different nutrient regimes may have only negligible effects on AMF colonisation, when the degree of AMF colonisation is optimal, such regimes can be influential in developing the flavour characteristics of apple and cherry fruit.

Key words: Mycorrhizal, EM, conventional, TSS, alternative, malic acid.

6.2 Introduction

Fertiliser programs currently used in conventional fruit and vegetable production aim to maximise crop yields; however, this can come at the expense of crop quality (Copetta *et al.*, 2011). Thus, there is an increasing interest in the adoption of alternative practices, which use organic matter and bio-fertilisers to reduce reliance on synthetic fertilisers, while still maintaining productivity. In line with this alternative movement, increasing interest has also been shown in the effects of the symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and the roots of plant hosts on plant growth and development (Johnson *et al.*, 1997, Jones and Smith, 2004, Moore *et al.*, 2015) However, to date, little research on this subject has been conducted in relation to fruit trees. Miller *et al.* (1985a) found that inoculation with AMF species, including *Glomus mosseae, G. maculosum, G. manihotis, G. bitunicatum* and *G. occultum,* in low-P soils was accompanied by an increase in height of apple seedlings, while in high-P soils, inoculation with AMF species such as *G. maculosum* and *Gigaspora calospora*, resulted in an increase in stem diameter.

Numerous studies have reported that AMF have the ability to capture nutrients from the soil, and transfer and deliver them to the host plant under a range of environmental conditions (Hattingh *et al.*, 1973, Ames *et al.*, 1983, George *et al.*, 1992, Smith *et al.*, 2000, Wang *et al.*, 2002). The role played by AMF in enhancing the productivity of the host plant may be affected by the degree of AMF colonisation, which in turn can be reduced or increased according to the levels of available mineral nutrients in the soil, especially P (Verkade and Hamilton, 1983, Koide, 1985, Thompson, 1987, Johnson, 2010). In addition, mycorrhizal activity has been shown to be influenced by the microbial rhizosphere community (Higa and Wididana, 1991, Bajwa *et al.*, 1999b, Barea *et al.*, 2005). Apart from the resident microbes of any soil, inoculation with other micro-organisms, such as "effective microbes" (EM) may influence AMF activity. Typically, these EM are commercial cultures containing up to 80

species of coexisting beneficial microorganisms, consisting of lactic acid bacteria, yeasts, photosynthetic bacteria and actinomycetes (Higa and Parr, 1994). According to Bajwa *et al.* (1999a) they have the ability to increase crop productivity by N-fixation, increasing photosynthesis and accelerating decomposition of lignin material in the soil (Bajwa *et al.*, 1999b). Several studies examining the effects of EM inoculation on AMF colonisation in a number of annual crops, such as pea, chickpea, soybean, maize and wheat, have shown that the different crops react differently to EM inoculation. Bajwa *et al.* (1999b) reported that growth of maize, soybean, chickpea and wheat increased as a result of increased AMF colonisation by EM. Another study noted that pea growth, yield and nodulation enhancement by EM inoculation was accompanied by a marked suppression of the AMF colonisation (Bajwa *et al.*, 1999b).

Soil amendments can improve the physical and chemical properties of soil, including water retention, soil density, total porosity, soil pH, and soil carbon content (Zimmerman, 2010, Hardie *et al.*, 2014, Abujabhah *et al.*, 2016) and play an influential role in promoting the AMF relationships in plant roots. Specific amendments commonly used in agricultural systems include biochar (Hardie *et al.*, 2014). Additionally, cover crops can be used to achieve similar outcomes to soil amendments. However, limited research has been done on the influence of soil amendments and cover crops on mycorrhizal associations in tree crops, or on their impact on fruit quality.

Biochar as a soil amendment can influence the activity of AMF. In a review of mycorrhizal responses to biochar in soil, Warnock *et al.* (2007) proposed several mechanisms to explain how biochar can alter the total abundance and/or activity of mycorrhizal fungi in soils and in plant roots. They suggest that the addition of biochar could (1) result in changes in nutrient availability and/or the physico-chemical properties of the soil; (2) cause changes that are either beneficial or harmful to other soil microbes, such as phosphate solubilising bacteria (PBS) or mycorrhization helper bacteria (MHB); or (3) act as a refuge from hyphal grazers.

Cover crops have been shown to enhance AMF colonisation of the main crop thereby increasing P uptake (Kabir and Koide, 2000), which reduces the effects of high concentrations of P on AMF colonisation. In addition, the roots of the cover crops can maintain an appropriate network of mycorrhizal hyphae and spores (Dodd and Jeffries, 1986). In their research on cotton, Dabney *et al.* (2001) found that the quick growth and yield of cotton grown on soil planted with wheat as a cover crop was the result of the cotton roots

taking advantage of the network of mycorrhizal hyphae present in the wheat roots. Hence, it would appear that cover crops can enhance mycorrhization, and indirectly provide a source of AMF inoculum for the main crop.

Fruit quality is the result of a combination of visual, textural and flavour characteristics. (Crisosto *et al.*, 2003) reported that several criteria of quality, including a high concentration of total soluble solids and titratable acidity, have been linked to consumer preference for particular fruit tree crops. For example, the ratio of TSS to titratable acidity (TA) at harvest plays a significant role in consumer demand for Bing cherries (Drake *et al.*, 1989, Cliff *et al.*, 1995, Dever *et al.*, 1996, Kappel *et al.*, 1996). Thus, fruit quality is one of the most important management considerations for horticultural producers, and further research is required to investigate how the interactions between organic amendments and mycorrhizal colonisation can improve fruit quality outcomes.

The present study consists of two components: firstly, the effect of nutrient regime in conjunction with soil amendments (biochar and cover crops) was investigated on the degree of AMF colonisation in apple roots growing in different soil type compared to cherries. Secondly, the effect of different nutrient regimes in combination with EM inoculation on AMF colonisation was investigated in cherry roots growing in two different soil types. In both components, the relationship between AMF colonisation and fruit quality criteria was studied through correlation analysis. Finally, the influence of nutrient regime and soil amendment treatments on crop leaf and soil nutrition in both cherry and apple orchards was examined.

6.3 Materials and methods

6.3.1 Experimental locations

Three trials were established over a six month period from spring (October) 2012 to autumn (March) 2013 in three established commercial orchards in southern Tasmania. The first trial was established using the apple cultivar ‘Royal Gala’ at Lucaston in the Huon Valley (42° 59’38.3” S, 147° 03’31.5” E). The second trial was established using ‘Sweetheart’ sweet cherry at Rosegarland in the Derwent Valley (42° 42’17.0” S, 146° 57’05.4” E), and the third trial on ‘Lapin’ sweet cherry at Nicholls Rivulet in the Huon Valley (43° 09’50.1” S, 147° 07’28.0” E). Orchard characteristics are outlined in Table 6.1.

Table 6.1 Details of each cultivar and orchard layout for the three sites.

Crop	Cultivar	Rootstock	Row orientation	Tree spacing	Planting date	Soil type
Apple	'Royal Gala'	M26	NE-SW	1 m x 4 m	2005	Sandy loam
Sweet cherry	'Lapin'	Colt	N-S	1.8 m x 4 m	2008	Loam
	'Sweetheart'	Colt	E-W	2 m x 5 m	2007	Dolerite/clay

6.3.2 Trial design and treatment application

Trial design at each site was a randomised complete block with four replicates of each treatment. Plot size for each replicate was five trees in a row section, with the centre tree used for assessment. The soil treatments applied in each trial are listed in Table 6.2.

Table 6.2 Treatments applied at each site. CC = cover crop of clover and grass

	'Royal Gala' apple	
	Nutrient regime	**Floor management**
CONV + HERB	Conventional	Herbicide
CONV + BC	Conventional	Biochar
CONV + CC	Conventional	Clover/grass
ALT + HERB	Alternative	Herbicide
ALT + BC	Alternative	Biochar
ALT + CC	Alternative	Clover/grass
	'Lapin' and 'Sweetheart' sweet cherry	
	Nutrient regime	**Effective microbes (EM)**
CONV	Conventional	(-)
CONV + EM	Conventional	(+)
ALT	Alternative	(-)
ALT + EM	Alternative	(+)

The conventional regime was a synthetic fertiliser program based on the commercial practice used in the orchard including herbicide application (Basta) twice per year. The alternative regime was a soil conditioner sold as FF 50 Bio-humate (Ferbon®, Bacchus Marsh, Australia) or compost blended with targeted minerals (see Tables 6.3 and 6.4 for full details). Ferbon was applied at the rate of 300 kg/ha, compost at 800 kg ha^{-1} with soluble humate granules (water soluble potassium humate 75 %, solubility 85 % and particle size 0.5 - 5 mm (> 90 %)) at 20 kg ha^{-1}.

Biochar was obtained from Pacific Pyrolysis, Somersby, NSW (Australia) and consisted of Acacia whole tree green waste which had undergone pyrolysis in a continuous flow kiln at temperatures up to 550 C° for between 30 and 40 min. The biochar had a pH of 6.4 with nutrient concentrations of 8.93% organic C, 3 mg kg^{-1} NH_4^+, 1 mg kg^{-1} NO_3^-, 234 mg kg^{-1} extractable phosphorus and 1117 mg kg^{-1} potassium. Biochar additions were applied at 5 kg per tree space, spread evenly across the soil surface and lightly raked into the topsoil (equivalent to 12,500 kg ha^{-1}).

As no understorey plants were growing under the apple trees due to herbicide applied in the previous seasons, white clover (*Trifolium repens*) and creeping red fescue grass (*Festuca rubra*) were germinated in a glasshouse and transplanted under the trees in the conventional + cover crops and alternative + cover crops treatment plots at a population density of 4 plants m^{-2} white clover and 6 plants m^{-2} creeping red fescue grass.

The effective microbes (EM) product was purchased as EM1 (Vital Resource Management (VRM) Pty Ltd., Qld). The product was activated by brewing in a 30 L fermentation vat under anaerobic conditions. Stock solutions were prepared by adding 30 ml EM1 and 30 ml molasses per litre of de-chlorinated water. This was left to brew for at least one week at ambient conditions in the glasshouse. EM was applied monthly at a rate of 75 mL of activated EM solution and 5 g of Acadian soluble seaweed extract (SSE) in 10 L of non-chlorinated water for each plot. All soil EM applications commenced at the start of the 2012-13 season.

Table 6.3 The typical analysis for organic applications used in the trial and amounts of targeted minerals added with alternative regime.

	N (mg/kg)	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Cl (mg/kg)
Compost	15000	7500	6100	2600	13100	9800	1800	18100	3700
Ferbon	13400	1990	4620	17700	14800	2790	2460	9100	NA

	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	B (mg/kg)	Mo (mg/kg)	PH	Electrical Cond.
Compost	383.3	199.9	65.1	6.7	30.8	4.4	6.5	2000 uS/cm
Ferbon	488.0	142.0	91.0	11.8	54.7	9.0	6.2	3255 uS/cm
Ferbon	Org. C 37.5 % and Moisture Con. 35.4 %							

Table 6.4 The composition of alternative nutrient regime used in apple ('Royal Gala') and cherry trials ('Sweetheart' trial established in October 2012 and 'Lapin' trial in March 2013).

	Amounts added by kg ha^{-1}							
	'Royal Gala' apple			**'Lapin' cherry**		**'Sweetheart' cherry**		
	Oct 2012	**May 2013**	**Sep 2014**	**May 2013**	**Sep 2014**	**Oct 2012**	**Aug 2013**	**Sep 2014**
Ferbon	300	300	--	300	--	300	300	--
Humified compost	--	--	800	--	800	--	--	800
Soluble humate granules	--	--	20	--	20	--	--	20
Dolomite lime (autumn)	--	--	--	--	--	--	--	500
Diatomaceous earth	--	--	1000	--	1000	--	--	--
Calcitic lime				--	1000	--	--	--
Gypsum	--	--	--	--	700	500	500	--
Elemental sulphur	80	80	55	50	--	--	--	--
Ammonium sulphate	50	50	--	50	--	30	30	--
Potassium sulphate	10	10	10	10	--	20	20	--
Manganese sulphate	20	20	--	20	25	25	25	1.5
Zinc sulphate	--	--	--	--	--	2	2	--
Copper sulphate	15	15	--	--	--	2	2	--
Borax	10	10	--	10	--	8	8	--
Sodium molybdate	0.5	0.5	--	0.5	--	0.5	0.5	--

Soil and leaf sampling

Soil samples for nutrient analysis were collected prior to the start of the trial and again at the end of the trial from around the centre tree in each plot using a spiral auger (Figure 1). Samples were collected at 0-10 cm depth at a distance of 15-30 cm from the trunk of each tree. The samples from each block were amalgamated to make one composite sample for each treatment. At the end of the trials leaf samples for nutrient analysis were collected by selecting 3-4 fully expanded leaves from each sample tree in each plot. Samples from each block were combined to make one composite sample for each treatment. Soil and leaf samples were sent to the Environmental Analysis Laboratory at Southern Cross University, Queensland, for nutrient analysis.

Figure 6.1 Collecting soil samples for nutrient analysis (spring).

6.3.3 Fruit material and laboratory analysis

Apple fruit were harvested in March 2014 and cherries in January 2014 aligning with commercial harvest. A total of 30 mature fruit were selected at random from the centre tree in each plot. Selected fruit were placed in plastic bags and stored at 0 °C overnight and laboratory assessments conducted the following day. Apple fruit were cut into quarters and juiced in an electric juicer. The juice was filtered through 45 micron filter paper. Cherry fruit were juiced by hand using a nylon filter cloth with a pore size of 100 microns. Juice samples were used for analysis of TSS and TA. An Atago PR-1 digital refractometer was used to determine TSS concentration while TA was measured on a 10 ml juice sample using a 702 SM Titrina, Metrohm titrator, and MA content calculated.

6.3.4 Mycorrhizal detection

For assessment of mycorrhizal colonisation, fine root samples were collected from the top 60 cm of the soil profile and at 30 - 45 cm distance from each tree trunk, from each of the five trees in each plot in spring 2013 (October), and summer (February), autumn (March) and spring (October) 2014. After collection samples were placed in labelled plastic bags, covered with soil from the plot and taken to the laboratory where they were prepared for mycorrhizal assessment.

Root samples were washed with tap water, placed in 50% ethanol (v:v) and stored at 4 °C. Stored roots were then prepared for colonisation estimation. Roots were rinsed with tap water, cut into 1–1.5 cm segments, placed in 100 mL glass, screw-cap Schott bottles and covered with 5% KOH. The Schott bottles were left at room temperature overnight, and the following day placed in a water bath at 85 - 90° C for 5 to 7 minutes. After heating, roots were drained and rinsed twice with tap water, rinsed in 7.5% hydrochloric acid (HCl) and returned to the rinsed Schott bottle. Root samples were stained with 5% black ink (schaeffer) in lactic acid (Khaosaad *et al.*, 2006, Toussaint *et al.*, 2007) and reheated for 3 to 5 minutes. Roots were then drained, rinsed once with tap water and placed in water with a few drops of lactic acid to destain.

To estimate AMF colonisation, the method of McGonigle *et al.* (1990) was modified using gridded slides. Five slides with five stained roots per slide mounted in water were prepared for each treatment. Using the modified gridded slide method, the crosshair eyepiece was replaced with gridded slides (twin grids 20 mm x 20 mm each with 1 mm line spacing) (Figure 6.2). AMF colonisation was scored by inspecting six intersections of each root with grid lines and presence of hyphae, vesicles and arbuscules recorded. With five roots per slide and five slides per replicate, there were a total of 150 intersections per replicate. The following formulae were used to calculate colonisation.

Hypha, vesicle or arbuscule presence = $\frac{X}{150} x100$

Where (X) = hyphae, vesicle or arbuscules

AMF colonisation = $\frac{Y}{150} x100$ where (Y) = any of any hyphae, vesicle or arbuscules

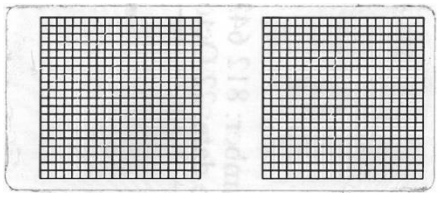


Figure 6.2 Gridded slide used to estimate AMF colonisation.

6.3.5 Statistical analysis

SAS Enterprise (version 6.1 M1HF5 (6.100.0.4180) 2013 by SAS Institute Inc., Cary, NC, USA) was used for data analysis. Colonisation data were analysed by ANOVA: Linear Models; nutrient regimes (conventional and alternative) and orchard floor (herbicide, biochar and cover crops) management applications considered as fixed factors for apples; or EM

inoculation (plus and minus) and fertiliser treatment conventional and alternative) considered as fixed factors for cherries. Data of AMF colonisation were transformed into LOG (x + 1) only when necessary for normality distribution. Treatment means were determined and the separation of means was based upon least significant difference (Fisher LSD). Pearson's product-moment correlation (Multivariate) was used to analyse correlation data between variables. Significance was considered at *P prob* < 0.05. SigmaPlot 12.5 (Systat Software, Inc.) was used to draw graphs.

6.4 Results

6.4.1 Mycorrhizal colonisation

The expected colonisation structures for AMF, including hyphae, vesicles and arbuscules, were found in both apple and cherry roots (Figure 6.3).

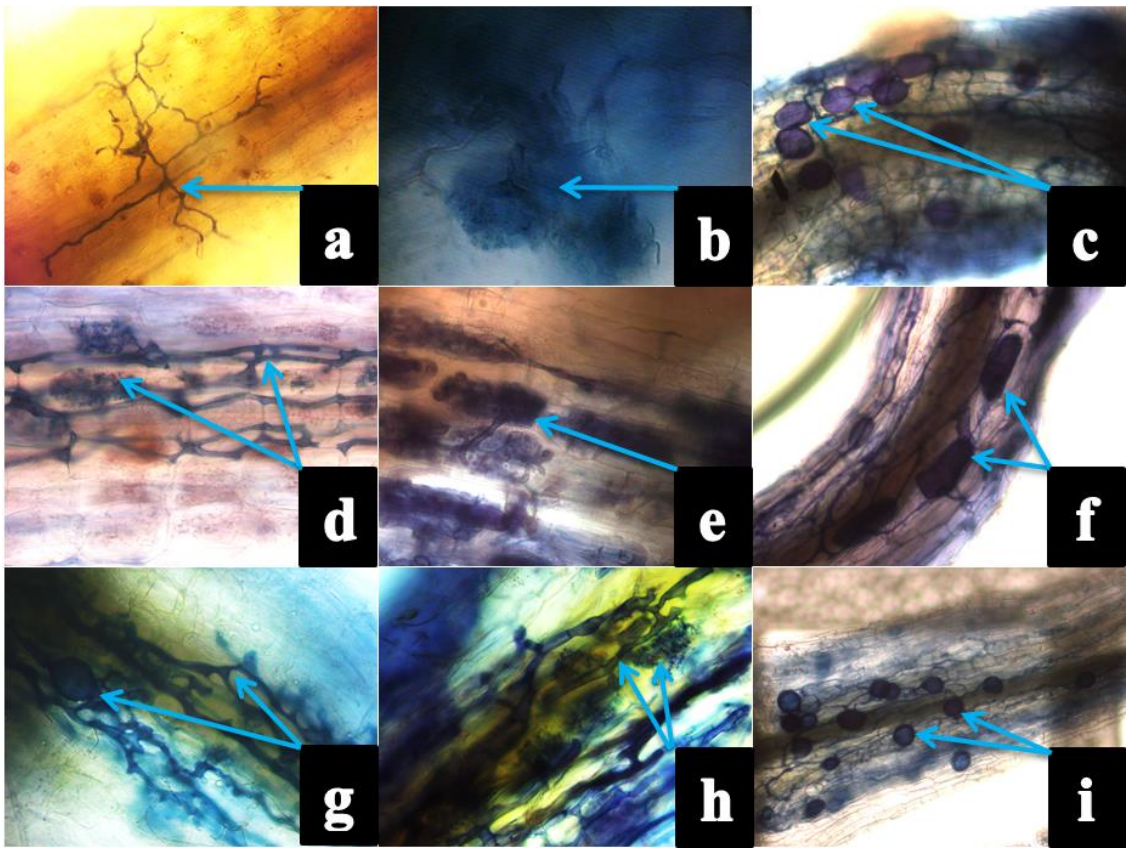


Figure 6.3 a) Hyphae in 'Royal Gala' apple roots, **b)** arbuscules in 'Royal Gala' apple roots, **c)** vesicles in 'Royal Gala' apple roots, **d)** arbuscules with hyphae in 'Lapin' cherry roots, **e)** vesicles in 'Lapin' cherry roots, **g)** vesicles with hyphae in 'Sweetheart' cherry roots, **h)** arbuscules with hyphae in 'Sweetheart' cherry roots and **i)** vesicles in 'Sweetheart' cherry roots.

Apple roots: There were no significant interactions between nutrient regimes and orchard floor management on AMF colonisation on all assessment dates (see Appendices Table 9.4 and 9.5). Examination of the main effects (Table 6.5 and 6.6) had no effect on AMF

colonisation in all assessment seasons under nutrient regime. Compared with herbicide and cover crops, biochar significantly increased the abundance of hyphae, vesicles and arbuscules in all assessment seasons, and also increased total AMF colonisation, with the exception of autumn 2014 (Table 6.6). Under all treatments, no arbuscules were detected in the autumn of 2014, though low levels were evident in the following spring. There was a large increase in the formation of vesicles in spring 2014 under all treatments.

Table 6.5 Mean presence of AMF colonisation structures in apple roots at different seasons of the year, subject to effects of (i) nutrient regime (ALT and CONV) and (ii) orchard floor management (HERB, CC and BC).

	Spring - 2013							
	H	**H LOG(x+1)**	**V**	**V LOG(x+1)**	**A**	**A LOG(x+1)**	**AMF**	**AMF LOG(x+1)**
				(i) Nutrient regime				
CONV	29.8	1.4	16.0	1.1	16.7	1.1	30.0	1.4
ALT	23.2	1.3	11.0	1.0	9.4	0.9	23.3	1.3
L.S.D.	*ns*	*ns*	*ns*	*ns*	*6.47*	*ns*	*ns*	*ns*
F prob.	*0.15*	*0.53*	*0.09*	*0.15*	*0.03*	*0.18*	*0.15*	*0.53*
				(ii) Orchard floor mgt.				
HERB	17.9 b	1.2 b	7.8 b	0.9 b	8.1 b	0.9 b	18.2 b	1.2 b
BC	38.8 a	1.6 a	23.2 a	1.3 a	19.1 a	1.2 a	39.0 a	1.6 a
CC	22.8 b	1.3 b	9.5 b	0.9 b	12.0 ab	1.0 ab	22.8 b	1.3 b
L.S.D.	*11.11*	*0.18*	*7.39*	*0.19*	*7.93*	*0.22*	*11.25*	*0.18*
F prob.	*0.002*	*0.005*	*<0.001*	*<0.001*	*0.03*	*0.03*	*0.002*	*0.005*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 6.6 Mean presence of AMF colonisation structures in apple roots at different seasons of the year, subject to effects of (i) nutrient regime (ALT and CONV) and (ii) orchard floor management (HERB, CC and BC).

	Autumn - 2014				Spring - 2014			
	H	**V**	**A**	**AMF**	**H**	**V**	**A**	**AMF**
				(i) Nutrient regime				
CONV	26.0	53.6	nd	63.2	55.0	48.0	4.0	65.8
ALT	22.3	51.0	nd	54.5	44.2	46.1	2.8	60.3
L.S.D.	*ns*	*ns*	-	*ns*	*ns*	*ns*	*ns*	*ns*
F prob.	*0.34*	*0.44*	-	*0.22*	*0.16*	*0.67*	*0.44*	*0.26*
				(ii) Orchard floor mgt.				
HERB	19.0 b	46.4 b	nd	60.1	47.0	39.8 b	2.8 b	56.9 b
BC	32.7 a	58.0 a	nd	60.0	52.9	60.3 a	6.7 a	73.8 a
CC	20.8 b	52.6 ab	nd	56.5	49.0	41.0 b	0.7 b	58.4 b
L.S.D.	*9.65*	*8.54*	-	*ns*	*ns*	*11.32*	*3.60*	*11.97*
F prob.	*0.02*	*0.03*	-	*0.89*	*0.80*	*0.002*	*0.009*	*0.015*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant, nd = not detected. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Cherry roots ('Lapin'): There were no interactions between nutrient regime and EM, on presence of hyphae (Appendices Table 9.6 and 9.7). The presence of arbuscules in spring 2014 was significantly greater under the alternative + EM treatment compared with the conventional + EM treatment combinations (Fig 6.4a). There was a significant interaction between nutrient regime and EM for vesicle abundance and AMF colonisation during summer 2014. At this time, both the presence of vesicles (Fig 6.4b) and total colonisation (Fig 6.4c) were lower under the alternative - EM compared with conventional - EM treatment. However, the addition of EM increased both variables under the alternative regime, while decreasing them under the conventional regime. No treatment effect was observed in the spring of either 2013 or 2014.

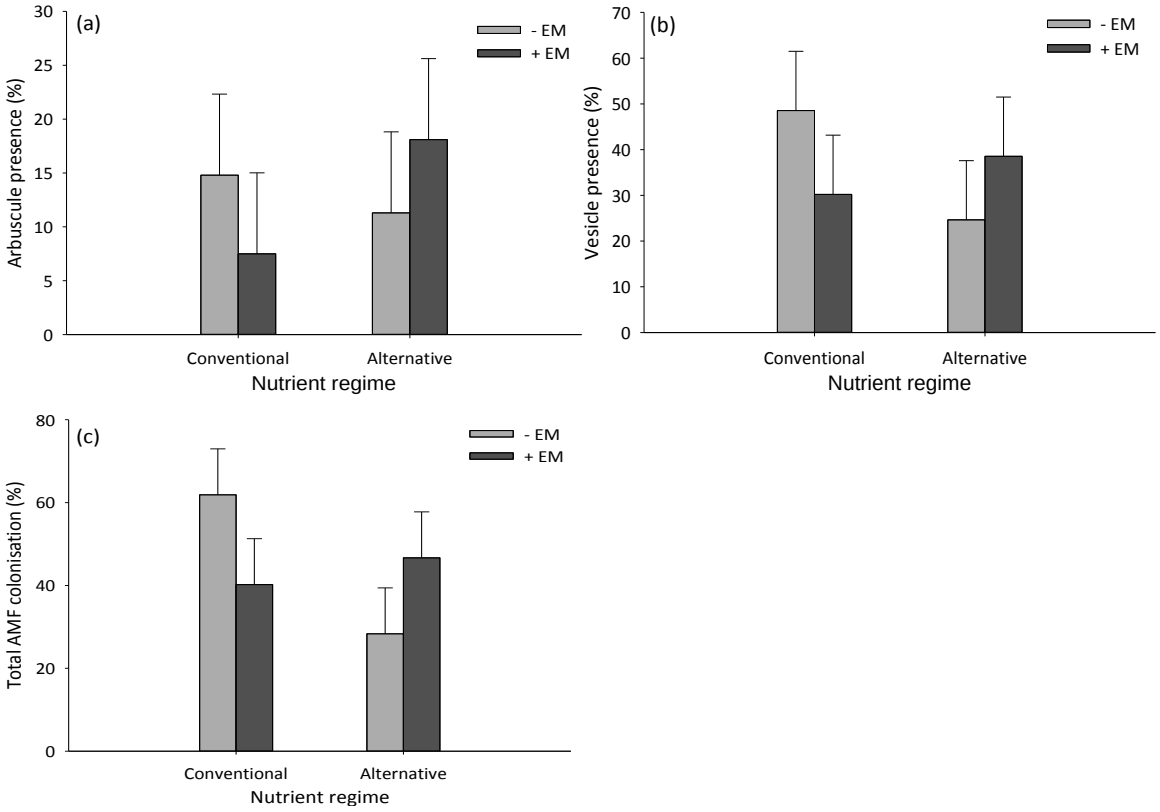


Figure 6.4 Mean presence of AMF colonisation structures in cherry roots (CV: 'Lapin', rootstock: Colt) at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (- EM and + EM) on (a) arbuscule presence (spring 2014), (b) vesicle presence (summer 2014) and (c) AMF colonisation (summer 2014).

Nutrient regime had no effect on AMF colonisation in spring 2013 (Table 6.7). Compared with the conventional regime, vesicles were significantly more abundant under the alternative regime in spring 2014 but significantly lower in summer 2014. Inoculation with EM resulted in a decrease in the numbers of vesicles and arbuscules in spring 2013, but had no effect on AMF colonisation in either spring or summer 2014 (Table 6.7). Under all treatments, both the

number of spores and total AMF colonisation continued to increase with the passage of the seasons.

Table 6.7 Mean presence of AMF colonisation structures in cherry roots (CV: 'Lapin', rootstock: Colt) at different seasons of the year, subject to effects of (i) nutrient regime (ALT and CONV) and (ii) effective microbes (- EM and + EM).

	Spring - 2013				**Summer - 2014**				**Spring - 2014**			
	H	**V**	**A**	**AMF**	**H**	**V**	**A**	**AMF**	**H**	**V**	**A**	**AMF**
					(i) nutrient regime							
CONV	40.0	16.2	11.5	42.6	36.4	39.4	7.9	51.04	55.8	38.1 b	11.1	59.8
ALT	28.4	15.7	6.7	28.5	22.6	30.2	2.7	37.5	59.7	52.1 a	14.7	67.1
L.S.D.	*ns*	*ns*	*ns*	*ns*	*ns*	*6.17*	*ns*	*11.09*	*ns*	*10.13*	*ns*	*ns*
F prob.	*0.22*	*0.92*	*0.13*	*0.16*	*0.07*	*0.01*	*0.08*	*0.02*	*0.58*	*0.01*	*0.17*	*0.28*
					(ii) Effective microbes							
(-) EMs	42.0	19.8 a	14.2 a	44.3	28.4	36.6	6.5	45.1	62.7	50.2	13.0	68.8
(+) EMs	26.6	12.0 b	3.9 b	26.8	30.5	34.4	4.1	43.4	52.8	40.7	12.8	58.1
L.S.D.	*ns*	*6.810*	*6.59*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
F prob.	*0.11*	*0.04*	*0.006*	*0.09*	*0.77*	*0.60*	*0.40*	*0.74*	*0.17*	*0.18*	*0.93*	*0.12*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Cherry roots ('Sweetheart'): There was a significant relationship between nutrient regime and EM in terms of arbuscule abundance in spring 2014 (Fig 6.5a). The addition of EM significantly increased the number of arbuscules under the conventional regime, but not under the alternative regime. In summer 2014, vesicle presence and total colonisation were significantly higher both under the conventional + EM and alternative - EM treatments compared to other treatments (Fig 6.5b). The addition of EM resulted in an increase in the number of vesicles and total AMF colonisation under the conventional regime, but a decrease under the alternative regime (Fig 6.5 b and c).

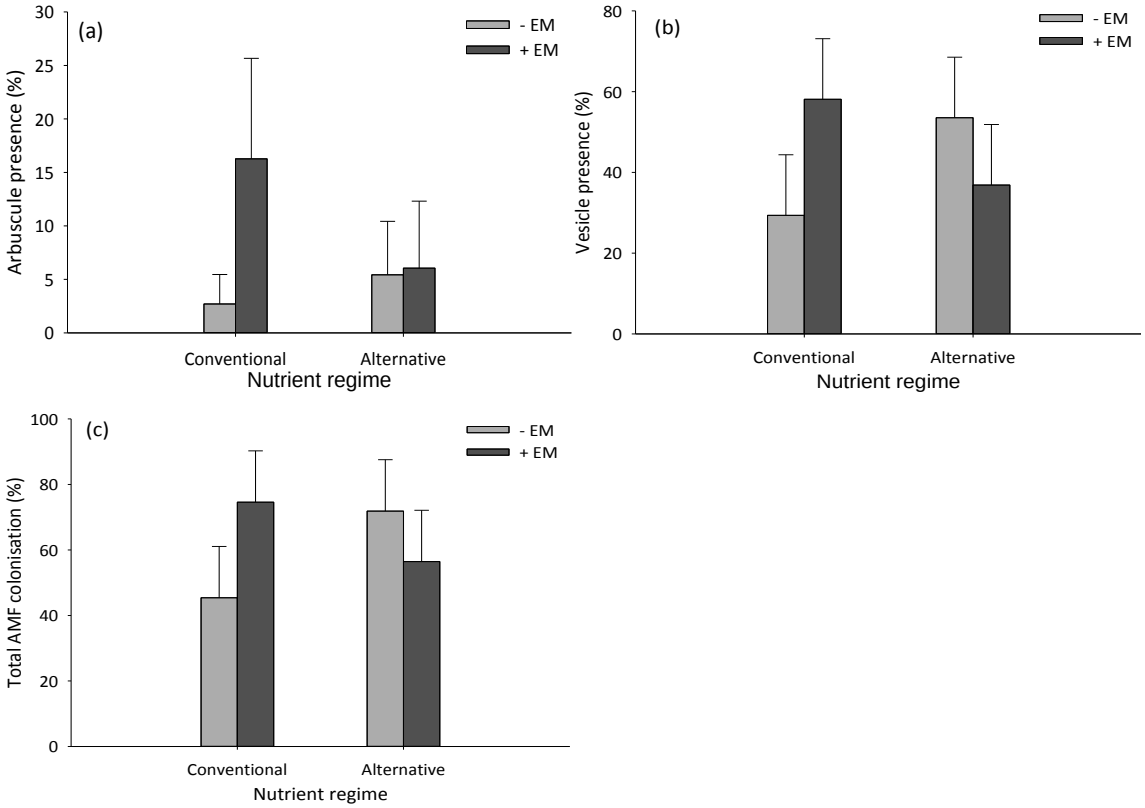


Figure 6.5 Mean presence of AMF colonisation structures in cherry roots (cv. 'Sweetheart': rootstock: Colt) at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (- EM and + EM) on (a) arbuscule presence (spring 2014), (b) vesicle presence (summer 2014) and c) total AMF colonisation (summer 2014).

Nutrient regime had no effect on AMF colonisation at any assessment time (Table 6.8). In the spring both of 2013 and 2014, EM inoculation was associated with increased formation of arbuscules, but, in spring, 2013, it resulted in a decrease in the number of vesicles (Table 6.8). There was no effect of treatment on AMF colonisation in summer, 2014 (Table 6.8 although the number of vesicles and total AMF colonisation was greater at that time, compared to spring, 2013 (Table 6.8).

Table 6.8 Mean presence of AMF colonisation structures in cherry roots (CV: 'Sweetheart', rootstock: Colt) at different seasons of the year, subject to effects of (i) nutrient regime (ALT and CONV) and (ii) effective microbes (- EM and + EM).

	Spring - 2013				Summer - 2014				Spring - 2014			
	H	**V**	**A**	**AMF**	**H**	**V**	**A**	**AMF**	**H**	**V**	**A**	**AMF**
					(i) nutrient regime							
CONV	42.3	19.8	4.4	42.6	45.0	43.8	9.7	60.0	39.5	28.7	9.5	39.9
ALT	57.7	15.8	7.0	57.8	51.5	45.2	12.1	64.2	49.1	23.9	5.7	49.4
L.S.D.	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
F prob.	*0.16*	*0.35*	*0.23*	*0.17*	*0.62*	*0.81*	*0.71*	*0.66*	*0.34*	*0.52*	*0.26*	*0.34*
					(ii) Effective microbes (EM)							
(-) EMs	49.0	23.6 a	2.7 b	49.4	48.2	41.5	6.3	58.6	32.5	24.2	4.1 b	47.9
(+) EMs	50.9	11.9 b	8.8 a	50.9	48.2	47.5	15.5	65.5	26.2	28.3	11.2 a	41.4
L.S.D.	*ns*	*8.693*	*4.403*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*6.11*	*ns*
F prob.	*0.85*	*0.02*	*0.01*	*0.88*	*1.00*	*0.34*	*0.16*	*0.48*	*0.53*	*0.58*	*0.04*	*0.50*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

6.4.2 Correlation of AMF with fruit quality

Apples: significant correlations were found between AMF colonisation and the flavour characteristics of TSS and TA, presented as MA, and the ratio of TSS/TA in apple fruit (Table 6.9). The TSS/TA ratio negatively correlated with AMF colonisation, while there was a positive correlation between both TSS content and TA level, and AMF colonisation and nutrient regime. However, the relationship between TSS and AMF colonisation was stronger under the alternative regime than under the conventional (*Correlation* = 0.72 and 0.53, respectively). In contrast, the correlation between TA and AMF colonisation was stronger under the conventional regime compared to the alternative (*Correlation* = 0.82 and 0.77, respectively).

An examination of the correlations with floor management treatments revealed that the AMF colonisation of roots, both under the herbicide and cover crops treatments, was positively correlated with TSS *(Correlation* = 0.75 and 0.63, respectively) and TA *(Correlation* = 0.79 and 0.83, respectively). However, for TSS/TA, only the cover crops treatment showed a correlation, and this was a negative one. Under the biochar treatment, there was no evidence of a correlation between AMF colonisation and any flavour characteristic.

Table 6.9 Pearson's product-moment correlation between AMF colonisation under different treatments and the development of the flavour characteristics (total soluble solids (TSS), titratable acidity (TA) as malic acid) and the ratio of TSS/TA) of apple fruit (Cv. 'Royal Gala').

			N	*F prob*	*Correlation (Cor)*
TSS	Overall		30	*<0.001*	0.58
MA (TA)	Overall		30	*<0.001*	0.69
TSS/TA	Overall		30	*0.004*	-0.51
TSS	Nutrient regime	CONV	15	*0.04*	0.53
		ALT	15	*0.003*	0.72
	Floor management	HERB	10	*0.02*	0.72
		BC	10	*0.10*	0.55
		CC	10	*0.04*	0.63
MA (TA)	Nutrient regime	CONV	15	*<0.001*	0.82
		ALT	15	*0.001*	0.77
	Floor management	HERB	10	*0.007*	0.79
		BC	10	*0.08*	0.57
		CC	10	*0.003*	0.83
TSS/TA	Nutrient regime	CONV	15	*<0.001*	-0.82
		ALT	15	*0.11*	-0.43
	Floor management	HERB	10	*0.09*	-0.55
		BC	10	*0.29*	-0.37
		CC	10	*0.03*	-0.69

Cherry fruit ('Lapin'): Overall, significant positive correlations were found between TSS content and AMF colonisation (P = 0.004), and TSS/TA and AMF colonisation (P = 0.04) (Table 6.10). The TSS values for fruit both under the conventional nutrient regime and minus EM were positively correlated to AMF colonisation (*Correlation* = 0.85 and 0.87, respectively). However, there was no correlation between MA and AMF colonisation, and while there was a correlation between TSS/TA and AMF colonisation, no significant correlations were observed for individual main effects.

Table 6.10 Pearson's product-moment correlation between AMF colonisation under different treatments and the development of the flavour characteristics (total soluble solids (TSS), titratable acidity (TA) as malic acid) and the ratio of TSS/TA) of cherry fruit (Cv. 'Lapin').

			N	*F prob*	*Correlation (Cor)*
TSS	Overall		16	*0.004*	0.67
MA (TA)	Overall		16	*0.94*	0.02
TSS/TA	Overall		16	*0.04*	0.50
TSS	Nutrient regime	CONV	8	*0.008*	0.85
		ALT	8	*0.30*	0.42
	Effective microbes	- EM	8	*0.005*	0.87
		+ EM	8	*0.13*	0.58
MA (TA)	Nutrient regime	CONV	8	*0.72*	0.15
		ALT	8	*0.16*	-0.54
	Effective microbes	- EM	8	*0.89*	0.06
		+ EM	8	*0.89*	-0.06
TSS/TA	Nutrient regime	CONV	8	*0.43*	0.32
		ALT	8	*0.08*	0.66
	Effective microbes	- EM	8	*0.26*	0.45
		+ EM	8	*0.09*	0.64

Cherry fruit ('Sweetheart'): 'Sweetheart' fruit showed a similar pattern to the 'Lapin' fruit in terms of the correlation between TSS and AMF, with a positive correlation overall, and also under the conventional nutrient regime ($P < 0.001$ and $P = 0.004$, respectively) (Table 6.11). However, unlike 'Lapin', the 'Sweetheart' fruit showed a significant positive correlation between MA and AMF colonisation under the conventional regime ($P = 0.007$), while under the alternative regime there was no correlation. Neither was there any correlation between TSS/TA and AMF colonisation.

Table 6.11 Pearson's product-moment correlation between AMF colonisation under different treatments and the development of the flavour characteristics (total soluble solids (TSS), titratable acidity (TA) as malic acid) and the ratio of TSS/TA) of cherry fruits (Cv. 'Sweetheart').

			N	*F prob*	*Correlation (Cor)*
TSS	Overall		16	*<0.001*	0.77
MA (TA)	Overall		16	*0.03*	0.53
TSS/TA	Overall		16	*0.51*	0.18
TSS	Nutrient regime	CON	8	*0.004*	0.88
		ALT	8	*0.07*	0.68
	Effective microbes	- EM	8	*0.01*	0.83
		+ EM	8	*0.12*	0.59
MA (TA)	Nutrient regime	CON	8	*0.007*	0.86
		ALT	8	*0.96*	-0.02
	Effective microbes	- EM	8	*0.17*	0.53
		+ EM	8	*0.22*	0.48
TSS/TA	Nutrient regime	CON	8	*0.83*	-0.09
		ALT	8	*0.29*	0.43
	Effective microbes	- EM	8	*0.24*	0.47
		+ EM	8	*0.64*	-0.20

6.4.3 Nutrient status in tree leaves

Nutrient levels varied between cultivars/sites, and in some instances between treatments (Table 6.12). In apple, N levels in leaves were lower under the biochar treatment. In the leaves of both cherry cultivars, the alternative + EM treatments produced the lowest N levels. In 'Sweetheart' cherry, the application of EM appeared to increase K in the leaves, while in 'Lapin' the conventional plus EM treatment produced the lowest leaf K level.

While there was little difference in leaf Ca content between treatments for apple leaves or for 'Lapin' cherry, in 'Sweetheart' cherry the highest level of leaf Ca was exhibited for the conventional treatment. Leaf Mg levels were relatively similar across treatments, but in 'Sweetheart' the Mg level appears to be higher under the conventional treatments than under either the alternative or both EM treatments.

Table 6.12 Nutrient status in leaves (each value in the table represents a composite sample from each treatment for all blocks), 20 June 2014.

Treatments	N (g kg-1)	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
			Apples ('Royal Gala')		
CONV	23.2	2.8	15.6	11.3	3.5
ALT	23.1	2.7	15.1	11.0	3.3
BC + CONV	22.5	2.8	15.4	10.8	3.2
BC + ALT	22.6	2.8	14.1	11.1	3.2
			Cherries ('Lapin')		
CONV	21.4	2.2	12.6	9.5	3.0
ALT	21.5	2.2	12.8	10.3	3.2
CONV + EM	21.7	2.2	12.2	10.6	3.1
ALT + EM	19.9	2.1	12.6	9.9	3.1
			Cherries ('Sweetheart')		
CONV	24.7	1.9	11.7	18.1	3.5
ALT	24.3	1.9	12.1	13.7	2.9
CONV + EM	25.0	1.8	12.6	14.9	3.1
ALT + EM	23.3	1.8	12.5	15.0	3.1

6.4.4 Nutrient status in the soil

Apple orchard: Compared to the baseline measurements taken at the beginning of the trial, the soil at the apple site (Table 6.13) showed a 15-35% increase in N whereas NH_4^+ -N was reduced slightly in all treatments, but a 30% reduction was observed under the biochar + alternative treatment. Nitrate N was reduced under the conventional and alternative treatments, but increased by 62% and 41% in the biochar + conventional and biochar + alternative, respectively. All treatments showed a two-fold increase in available P compared to the baseline, while K was reduced under the alternative treatment and increased with biochar. Mg increased by ~25% both under conventional and alternative treatments, and by 33-36% with the addition of biochar. Biochar additions increased Ca up to 15%, while there was no effect observed in Ca concentrations under the nutrient regimes. The level of S more than doubled under the alternative treatment, and increased by ~70% for all other treatments. Biochar application increased available K level by ~20%, and total C by more than 50%. There was negligible effect of treatment on available Ca. All treatments increased Zn, with the greatest effect produced by the alternative treatments. Compared to the baseline value, Mn concentrations decreased under all treatments, with the lowest values being observed

with BIO additions. All treatments resulted in a 2-6% reduction in soil pH. Compared to the baseline measurement, biochar + conventional and biochar + alternative treatments both caused an increase in EC by 0.012 and 0.003 units, respectively, while with conventional and alternative treatments, there was a decrease in EC by 0.04 and 0.033 units, respectively.

Table 6.13 Nutrient status in the soil (each value in the table represents a composite sample from each treatment for all blocks), apple orchard, 0-10 cm depth.

Treatments	Total N (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Morgan P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	S (mg/kg)
Apple orchard soil – baseline measurements - 28 September 2012								
Baseline	0.20	8.5	6.1	31.8	214.5	181.2	2924	275
Apple orchard soil – 20 June 2014								
CONV	0.27	7.0	4.7	64.1	218.4	242.4	2924	476
ALT	0.26	7.0	5.6	60.8	179.4	241.2	2894	612
BC + CONV	0.23	6.7	9.9	67.6	237.9	285.6	3020	465
BC + ALT	0.26	5.8	8.6	65.0	214.5	271.2	3460	524
	Exc. K (meq/100g)	Exc. Mg (meq/100g)	Exc. Ca (meq/100g)	Zn (mg/kg)	Mn (mg/kg)	Total C (%)	Soil pH	EC (ds/m)
Baseline	0.42	3.75	21.83	14.1	39	2.51	7.60	0.189
CONV	0.41	4.88	21.15	18.4	13	3.84	7.30	0.149
ALT	0.35	4.92	21.25	22.2	14	3.79	7.10	0.156
BC + CONV	0.44	5.46	20.75	19.8	9	4.20	7.30	0.201
BC + ALT	0.35	4.67	21.38	23.0	12	4.91	7.40	0.192

* Chemical analysis of cover crop treatment is not included in the table due the farmer has applied herbicide before the end of the trial.

Cherry orchard ('Lapin'): In the 'Lapin' cherry orchard (Table 6.14), alternative, and conventional plus EM treatments showed a 32% increase in total N compared with the baseline; NH_4^+-N increased approximately three-fold under the alternative treatment. Nitrate N increased approximately six-fold with the conventional, and four-fold with the alternative, conventional plus EM and alternative plus EM treatments. There was marked change over time in the levels of available P. Concentration of K decreased by ~24 to 33% with EM inoculation, and also slightly decreased with the alternative treatment, but increased with the conventional management. While concentrations of Mg and Ca decreased by 19% with the alternative plus EM treatment, they were not greatly affected by the rest of the treatments. All treatments increased S, with the highest increase of ~43% occurring with the conventional plus EM treatment. The highest increases of available K, Ca and Zn were noted under the

conventional treatment, while highest total C occurred with the conventional plus EM. The amount of available Mg fell with all treatments, particularly those involving the application of EM. Both of the nutrient regimes and EM inoculation increased Mn concentration compared to the baseline value; the greater increase (two-fold) was noticed with the alternative + EM treatment. All treatments resulted in a decrease in soil pH. The conventional and alternative treatments both caused an increase in EC by 0.014 units, while the conventional plus EM and the alternative plus EM resulted in a reduction in EC by 0.01 and 0.015 units respectively.

Table 6.14 Nutrient status in the soil (each value in the table represents a composite sample from each treatment for all blocks), cherry orchard ('Lapin'), 0-10 cm depth.

Treatments	Total N (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Morgan P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	S (mg/kg)
Cherry ('Lapin') orchard soil – 4 April 2013								
Baseline	0.17	9.7	1.2	16.5	351.0	276.0	2546	226
Cherry ('Lapin') orchard soil – 20 June 2014								
CONV	0.20	9.0	5.9	40.6	374.4	255.6	2442	349
ALT	0.25	27.7	4.5	41.2	335.4	268.8	2600	396
CONV + EM	0.25	9.6	3.9	39.2	265.2	253.2	2344	398
ALT + EM	0.24	7.6	4.4	33.4	234.0	222.0	2042	395
	Exc. K (meq/100g)	Exc. Mg (meq/100g)	Exc. Ca (meq/100g)	Zn (mg/kg)	Mn (mg/kg)	Total C (%)	Soil pH	EC (ds/m)
Baseline	0.47	10.67	17.45	12.4	3	2.33	7.20	0.094
CONV	0.78	5.67	19.45	29.9	4	3.56	6.90	0.108
ALT	0.65	5.58	19.38	22.3	5	3.74	6.80	0.108
CONV + EM	0.55	5.54	18.45	22.9	5	3.90	6.50	0.084
ALT + EM	0.54	5.38	17.80	24.0	6	3.21	6.30	0.079

Cherry orchard ('Sweetheart'): In the 'Sweetheart' cherry orchard (Table 6.15), all treatments resulted in a ~3-6% increase in total N, except for the conventional plus EM treatment which resulted in a reduction in total N by 3% compared with the baseline. Ammonium-N increased by 17-31% with the nutrient regimes, but decreased by 16-19%, with the application of EM. All treatments decreased NO_3^--N, with the greatest reduction (four-fold) occurring with the alternative treatment. There was also a marked increase in levels of available P (~38-55%) in all treatments, except the alternative plus EM, which decreased P by ~2%. Furthermore, K concentration increased in all treatments. However,

there were slight decreases in Mg and Ca concentrations with all treatments, with the exception of the alternative + EM one, which resulted in a slightly higher concentration of Ca. Over time, various changes also occurred in the levels of S. All treatments increased available K, with the highest increase of ~47% being noted with the conventional treatment. Available Mg decreased in all treatments by 18-30%. Conventional and alternative + EM treatment resulted in the release of greater amounts of available Ca, by approximately 3-7%, while this availability decreased by 1-3% with both the conventional + EM and alternative treatments. All treatments caused an increase in total C and Zn with the highest increases, 16% for total C and 30% for Zn, occurring under the alternative treatment. All treatments resulted in a fall in Mn concentrations, with the smallest decrease of less than two-fold being observed in EM treatments. All treatments led to a decrease in the soil pH, except under the alternative treatment, where it increased. All four treatments resulted in a decrease in EC by 0.134, 0.105, 0.072 and 0.161 units, respectively.

Table 6.15 Nutrient status in the soil (each value in the table represents a composite sample from each treatment for all blocks), cherry orchard ('Sweethear't), 0-10 cm depth.

Treatments	Total N (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Morgan P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	S (mg/kg)
		Cherry ('Sweetheart') orchard soil – baseline - 28 September 2012						
Baseline	0.27	14.2	43.9	17.7	413.4	338.4	3454	374
		Cherry ('Sweetheart') orchard soil – 20 June 2014						
CONV	0.28	17.2	13.0	36.1	780	259.2	3346	351
ALT	0.29	20.7	10.8	39.7	659.1	284.4	3906	394
CONV + EM	0.26	11.8	25.1	28.9	542.1	232.8	3478	375
ALT + EM	0.28	11.5	13.4	17.3	686.4	307.2	3552	417
	Exc. K (meq/100g)	Exc. Mg (meq/100g)	Exc. Ca (meq/100g)	Zn (mg/kg)	Mn (g/kg)	Total C (%)	Soil pH	EC (ds/m)
Baseline	0.59	5.17	19.00	8.5	61	2.94	6.60	0.360
CONV	1.13	3.96	18.35	8.7	19	3.31	6.50	0.226
ALT	0.87	3.96	19.60	12.3	29	3.51	6.70	0.255
CONV + EM	0.79	3.58	19.25	11.5	35	2.93	6.50	0.288
ALT + EM	0.90	4.21	17.58	10.6	35	3.33	6.20	0.199

6.5 Discussion

6.5.1 Mycorrhizal colonisation

Results of this trial have demonstrated that, while a change from conventional to alternative nutrient practices may have no effect on AMF colonisation structures on the roots of apple trees, it can cause positive changes to these same structures when they are associated with cherry tree roots. The only exception to this is hyphal colonisation, which remained unaffected. It was noted, however, that the degree of change in AMF colonisation differed between the cherry cultivars, and there is a possible explanation for this. As each cherry trial was conducted in a different orchard, with different soil types, it is likely that, in the case of cherry orchards, the effects of nutrient management on AMF colonisation may be associated with the soil type and EM applications. Thus, these factors may have the greatest influence on AMF colonisation.

6.5.1.1 Interaction of nutrient regimes and soil amendments

***Apple orchard*:** Results of the apple trial generally indicated that nutrient regimes had no effect on total AMF colonisation during the trial period. This could be attributed to high P concentrations both in tree leaves and the soil. The results revealed that the P concentration in the former ranged between 0.27 - 0.28% of the dry weight. According to Reuter and Robinson (1997), the standard range of P in apple leaves is between 0.15 – 0.20% of the dry weight, therefore a range of 0.21 - 0.30% is considered high. Menge *et al.* (1978b) observed that P concentrations in plant leaves can change the colonisation of endo-mycorrhizal fungi. These results supported our findings, where high leaf P contents in nutrient regime treatments may have inhibited AMF colonisation in apple roots. Indeed, colonisation was relatively low in the spring of 2013 (< 51%) and only slightly higher in the autumn of 2014 (< 60%). Moreover, Menge *et al.* (1978b) reported that a higher soil concentration of P will not inhibit mycorrhizal colonisation if the concentrations of P in the roots is low. On the other hand, an earlier study of sour orange trees, by Menge *et al.* (1978a), found that mycorrhizal citrange spores decreased from 5.7 to 0 spores cm^{-3} soil when soil P concentration increased from 28 to 56 mg kg^{-1}. Results of the present study indicated that soil available P concentrations in the apple orchard rose by 53% over the critical concentration recommended by Peverill *et al.* (1999), and remained relatively stable between nutrient regimes. Consequently no differences were observed in AMF colonisation due to high concentrations of soil available P.

Mäder *et al.* (2000) reported that, when host plants were exposed to high levels of nutrients, they drastically limited or stopped altogether the provision of resources to their fungal symbiotic partners. The result of this was a reduction in mycorrhizal colonisation. This is confirmed by other studies that also noted that plants exposed to high concentrations of P are typically associated with decreased AMF colonisation (Koide, 1985, Baon *et al.*, 1992, Johnson, 2010, Cavagnaro, 2014). Furthermore, in a study on the effects of soil P levels on plant growth, Miller *et al.* (1985a) found that apple seedlings thrived when P levels in the soil were high, regardless of AMF activity. Miller *et al.* (1985b) found that the degrees of AMF colonisation on apple rootstock seedlings were negatively correlated with soil P and Zn levels. In the present study, Zn concentrations were extremely high in the apple orchard compared to the recommended adequate level of Zn concentration for apple of 4 mg/kg (Peverill *et al.*, 1999). Soil Zn in the current trial was between four to five-fold higher than the adequate level under all treatments and this may also have contributed to the lack of effect on AMF colonisation.

Results show that the formation of symbiotic associations between apple roots and AMF is controlled by nutrient levels, like that of P. Thus, orchard floor management can be manipulated to enhance these associations. For example, the addition of biochar, and planting of in-row cover crops generally appears to result in positive effects upon AMF colonisation. In the case of biochar, changes in AMF colonisation do not appear to be due to changes in the chemical characteristics of the soil, such as P content, but rather may result from changes in the microbial biomass in the soil. If these changes involve an increase of beneficial microbes, they will favour mycorrhizal colonisation; on the other hand, if the increase is of harmful microbes, the colonisation will decrease. Warnock *et al.* (2007) reported that biochar additions may lead to changes, either beneficial or harmful, in other soil microbes, such as PBS or MHB. These authors further propose that biochar acts as a refuge for hyphal grazers. With regard to cover crops, P-concentration in the soil may decline as a result of uptake by the cover crops. Kabir and Koide (2000) demonstrated that cover crops enhance AMF colonisation by increasing P uptake, thus reducing the negative effects of high concentrations of P on AMF colonisation. In addition, the roots of the cover crops can maintain an appropriate network of mycorrhizal hyphae and spores (Dodd and Jeffries, 1986, Dabney *et al.*, 2001). Hence, it appears that cover crops may provide a source of AMF inoculum, perhaps in the same way that the cover crops in the present study supplied the apple roots with a source of AMF colonisation.

6.5.1.2 Interaction of nutrient regimes and EM application

***Cherry orchards*:** Results showed clear evidence that the interaction between EM inoculation and nutrient regimes can increase or decrease root colonisation. The key to this interaction appears to be achieving appropriate levels of certain nutrients, such as P and Zn, in the soil. In the 'Lapin' cherry orchard, under both nutrient regimes Cowell P levels ranged between 33.4 to 41.2 mg kg^{-1}, while they were between 17.3 and 39.7 mg/kg, under the same treatments, in the 'Sweetheart' orchard. Both these concentrations were much lower that the critical (53 mg/kg) concentration recommended by Peverill *et al.* (1999). The Zn level in the 'Lapin' orchard was higher than the baseline sample, by about 2-fold, under the conventional minus EM treatment; this is also higher than the standard range of between 2 and 10 mg/kg. With regard to the 'Sweetheart' orchard, Zn levels were 11.5 mg/kg under the conventional plus EM and 12.3 mg/kg under the alternative minus EM, which were approaching the standard range Therefore, the increase in AMF colonisation in 'Lapin' roots under the conventional minus EM treatment may be attributed to the increased Zn level in the soil, as Zn affects AMF colonisation (Miller *et al.*, 1985b). On the other hand, the increase in AMF colonisation in 'Sweetheart' roots under conventional plus EM or alternative minus EM treatments may be attributed to the low Cowell P level in the soil.

6.5.1.3 Season effect

In addition to the impact of horticultural practices, results showed that total AMF colonisation and number of vesicles on the roots can vary greatly within any one year according to the seasons, as well as from year to year. Vesicle numbers on apple roots increased at the autumn 2014 sampling and on the roots of both cherry cultivars in the following summer. There are several possible reasons for this. Firstly, according to Purin *et al.* (2006), there are seasonal variations of sporulation for some AMF species. Schultz *et al.* (1999) state that this occurs because of morphological variation in *Glomales* (characteristics of the spore wall). For example, some isolates of *Glomus etunicatum, Acaulospora laevis, Acaulospora colossica* and *Paraglomus occultum* have greater sporulation during cold seasons. Whereas, *Glomus geosporum, Acaulospora bireticulata* and *Gigaspora gigantea* isolates have greater sporulation during warm seasons (Purin *et al.*, 2006). Thus, the observed decline in AMF sporulation in the first season might be a reaction to cultural practices, such as nutrient regimes and/or orchard floor management (changes in the soil temperature). This speculation is supported by other studies which found that some practices such as use of fertilisers, agricultural rotations and liming, can affect spore production (Mosse and Bowen,

1968, Abbott and Robson, 1977). Additionally, the increase in AMF colonisation and abundant vesicle formation in the cherry roots could be attributed to the fact that it is during the summer that cherry trees reach the peak of their physiological activity. In their study on apple trees, Purin *et al.* (2006) also found an increase in the number of spores in the roots during the summer. They concluded that such an increase may be related to the peak activity of apple trees that occurs in the summer, which provides a greater amount of the carbon required for AMF growth and reproduction.

6.5.2 Fruit flavour

All observations from these trials indicate that, in the presence of suitable mycorrhizal colonisation, a shift from the conventional nutrient regime to a renewable alternative nutrient regime can alter the ratios of flavour characteristics in apple and cherry fruit. These changes in flavour may be attributed to the varying availability of nutrients, such as K and N sources (NH_4^+ and NO_3^-). Nava *et al.* (2007) found that N and K are usually present in apple (*Malus x domestica Borkh*) fruit *in* higher concentrations than other macro-nutrients, and that it is these elevated concentrations that affect the quality of the fruit.

6.5.2.1 Apple fruits

Nutrient regimes: Results of this study revealed that TSS and MA were associated with the degree of total AMF colonisation in apple fruit. The highest content of TSS was observed under the alternative regime, while the highest content of MA was observed with the conventional. According to Reuter and Robinson (1997), N and K concentrations in tree leaves grown under both regimes attained an almost adequate level (adequate level for N being 2.0 - 2.4, and between 1.2 and 1.5 for K). Therefore, it might be fair to assume that N and K concentrations in fruit produced by both regimes in the present study might also be considered adequate. This would then explain why there were no negative or positive effects from the N and K concentrations. While foliar P and Mg were somewhat influenced by the nutrient regimes, the concentrations of these minerals under both regimes were one to two-fold higher than the adequate concentration range as defined by Reuter and Robinson (1997). However, foliar P and Mg concentrations were slightly lower in leaves of trees under the alternative regime compared to those treated with the conventional regime. This may explain the high MA content recorded under the conventional treatment. Moreover, the low TSS which coincided with this high MA reading may explain the resulting acidity of the fruit. This

is supported by Casero *et al.* (2004) who found that acidity of Golden Smoothee apples positively correlated with P, K, Mg and Ca nutrients, in both the fruit and leaf.

Orchard floor management: Results of the current trials also demonstrated that the MA content of fruit trees responded positively to management involving cover crops and herbicides. This could be due to the high nutrient concentrations delivered to the fruits by their trees. It is already well known that cover crops, especially legumes, have the ability to fix atmospheric N, which means more available N in the soil (Dabney *et al.*, 2001). Additionally, Hoagland *et al.* (2008) noticed that controlling weeds by ploughing them back into the soil reduced the completion for available N and other elements, resulting in satisfactory growth of the apple trees. In the current trial, controlling weeds with herbicides may have produced the same results.

The ratio of TSS/TA in this study was negatively associated with AMF colonisation both in conventional and cover crop treatments; this may be due to the higher concentration of MA relative to TSS, as explained above.

6.5.2.2 Cherry fruits

Nutrient regimes: Similar to apple fruits, in both cherry cultivars TSS content and MA level responded positively to the conventional nutrient regime. In 'Lapin', the results indicated that concentrations of N, K and Ca reached the marginal level in all treatments; however, the concentrations of both N and Ca were lower under the conventional regime than under the alternative. Moreover, total AMF colonisation was reduced in the conventional regime, meaning that lower concentrations of nutrients, especially K, could be delivered to the tree roots by AMF. Therefore, the reason behind the improvement in TSS content in conventionally treated fruit may be their low N and Ca content (see the above explanation of the effects of high calcium content in fruits). This is supported by the finding of Nava *et al.* (2007) who found that TSS content in apple fruit is negatively correlated with N fertilisation, but positively influenced by K fertilisation. On the other hand, the improvement of TSS in fruit of the 'Sweetheart' cultivar may be due to the cultivar effect, as, according to Kappel *et al.* (2002) and Serradilla *et al.* (2012) cherry fruit quality, including TSS and MA content, can depend on cultivar .

Microbial (EM) inoculation: The results show that use of conventional practices, coupled with inoculation with EM is associated with a decrease in the TSS content of fruit. This may not be direct cause-effect relationship, because the results indicated that EM inoculation can

also cause a decline in AMF colonisation. Thus, it could be assumed that the absence of EM inoculation would lead to an increase in AMF colonisation and therefore, of nutrient uptake. This may explain the increase in the TSS content in trials using treatments that did not involve inoculation with EM.

6.5.3 Nutrient Status

6.5.3.1 Tree leaves

By the end of the trial period, both nutrient regimes had brought nutrient concentrations in apple leaves up into the standard range, while leaf P and Mg concentrations had exceeded the standard range, as specified by Reuter and Robinson (1997). This can be attributed to the fact that these minerals were added to the soil and so were in an available form for uptake by the tree roots. In contrast, the concentrations of leaf N, K and Ca in 'Lapin' and K in 'Sweetheart' fell to within minimum ranges as defined by Reuter and Robinson (1997). As each of the cherry trials was conducted in a different orchard, with a different soil type, it is likely that both the fertility and general chemical characteristics of the soil had a considerable influence on leaf nutrient status. After treatment applications, the soil in the 'Lapin' orchard had low concentrations of mineral nutrients and a high soil pH, compared to that of the 'Sweetheart' orchard, relative to the baseline level. Since Hyland *et al.* (2005) and Lyle *et al.* (2006) have shown that pH has a major influence on the availability of nutrients in the soil, perhaps the marginal level of N, K and Ca in 'Lapin' and K in 'Sweetheart' leaves that occurred in the present study could be attributed to the effects of a combination of these factors (soil fertility, chemical characteristics including soil pH and mineral nutrient concentrations).

Further, it is believed that the concentrations of leaf nutrients may not always reflect the correct nutritional status of trees due to nutrient dilution and diffusion that occurs, especially when trees have a large crown. This is confirmed by several studies that have demonstrated that the rapid growth of different types of natural vegetation in fertile soils causes nutrients in the plant tissues to diffuse and dilute, thereby reducing their concentration (Boyd and Hess, 1970, Harner and Harper, 1973, Auclair, 1977, El-Ghonemy *et al.*, 1978, Williams *et al.*, 1978).

6.5.3.2 The soil

The results of almost two years of apple trials show that there was a slight improvement in available levels of NO_3^--N, P, K, Mg, and total C and Zn in the soil, with biochar treatment.

There are several possible reasons for this: firstly, biochar can change soil nutrient availability, especially that of P, by altering the pH of the soil (Warnock *et al.*, 2010). This is supported by Major *et al.* (2009) who state that biochar additions affect nutrient availability and efficiency. Secondly, biochar also has the ability to decrease nutrient leaching. For example a study by Major *et al.* (2009) demonstrated that the addition of biochar decreased NH_4^+ leaching in the surface soil, and biochar derived from low nutrient timber improved the maintenance of N in soil. Furthermore, results indicated that the soil in the Lapin orchard was improved by the liberation of P, K, Exc. K and Ca in response to EM inoculation, and in both orchards by the release of Mg and Exc. Mg. EM inoculation also reduced the pH in the soil of both cherry orchards, which may have caused more nutrients to be released. Hyland *et al.* (2005) and Lyle *et al.* (2006) mentioned that soil pH plays a major role both in the release and absorption of nutrients from the soil.

6.5.4 Conclusion

This series of trials demonstrate that orchard floor management can affect the degree of AMF colonisation more than the shift from one nutrient regime to another. The results also showed that EM inoculation can have both positive and negative effects on AMF colonisation, depending on soil type. Another important finding was that the adsorption of certain nutrients, especially P, is dependent upon their availability in optimal concentrations in the soil. Finally, cover crops constitute good orchard floor management for the development of good quality fruit flavour. Thus, AMF colonisation, in conjunction with limited P input from orchard management practices, can have the potential to be a useful agricultural tool for the development of high quality fruit flavour characteristics.

"Chapter 7" General Discussion

To retain high levels of productivity, conventional farming systems need to use increasing amounts of synthetic fertilisers and pesticides, yet there is an increasing awareness of the impact of conventional agriculture on soil biology and the importance of soil biology in maintaining soil health and crop productivity. While the benefits of organic soil amendments and bio-fertilisers have been highlighted by many studies, outcomes for plant growth are not necessarily predictable due to the wide range of products available, the interactions between organic amendments and bio-inoculants, and their interactions with different soil types.

The objectives of the studies within this dissertation were to improve knowledge on the effects of organic nutrient regimes compared with conventional nutrient regimes on total plant growth. Pot grown sunflower plants were used as a model system to investigate the impact of different organic soil amendments (Chapter 3 - 5). The bio-inoculants studied included effective microbes (EM) and arbuscular mycorrhizal fungi (AMF). The impact of organic amendments, EM and orchard floor management on AMF colonisation was also examined in a 3-year study in commercial apple and cherry orchards (Chapter 6). Nutrient regime and inoculation with EM and AMF exerted a variety of effects on the growth and development of sunflower, AMF colonisation structures of sunflower, apple and cherry roots and on fruit flavour of cherry and apple fruits (See Table 7.1 to 7.4).

7.1 Summary of key results

The key results of each study presented in this dissertation are shown in Tables 7.1 – 7.4.

Table 7.1 Key findings of the trials presented in Chapter 3 for selected parameters. Treatments with significantly different values compared to other treatments are highlighted (↑ significantly increased, ↓ significantly decreased) and those not different are not shown.

Chapter	Treatments	Key findings
3 (sunflower trial 1 – basic potting mix)	1. CON -EM 2. CON +EM (15) 3. FER (150) -EM 4. FER (150 +EM (15) 5. FER (300) -EM 6. FER (300 +EM (15) 7. LIF (50%) -EM 8. LIF (50%) +EM (15) 9. LIF (100%) -EM 10. LIF (100%) +EM (15) 11. FER (150), LIF (50%) -EM 12. FER (150), LIF (50%) +EM (15) 13. FER (300), LIF (50%) -EM 14. FER (300), LIF (50%) +EM (15) 15. FER (150), LIF (100%) -EM 16. FER (150), LIF (100%) +EM (15) 17. FER (300), LIF (100%) -EM 18. FER (300), LIF (100%) +EM (15) 19. - EM, Main effect 20. + EM (15) Main effect 21. Nil FER 22. FER (150), Main effect 23. FER (300), Main effect 24. Nil LIF 25. LIF (50%), Main effect 26. LIF (100%), Main effect	**Plant height** ↑ #20 (w6-9), #23 (w7), #26 (w6-9) **Stem diameter** ↑#7, #8, #9, #10, #20, #25, #26, **Flowering time** ↓ #20, #23 **Flower head diameter** ↑ #8, #9, #13, #15, #22, #25, #26 ↓ #23 **Flower number** ↑ #7, #8, #10, #20, #26
3 (sunflower trial 2 – basic potting mix)	1. CON -EM 2. CON +EM (15) 3. CON +EM (30) 4. FER (450) -EM 5. FER (450) +EM (15) 6. FER (450) +EM (30) 7. LIF (100%) -EM 8. LIF (100%) +EM (15) 9. LIF (100%) +EM (30) 10. FER (450), LIF (100%) -EM 11. FER (450), LIF (100%) +EM (15) 12. FER (450), LIF (100%) +EM (30) 13. - EM, Main effect 14. + EM (15), Main effect 15. + EM (30), Main effect 16. CON (FER, main effect) 17. FER (450), Main effect 18. CON (LIF, main effect) 19. LIF (100%)	**Plant height** ↑ #2 (w6, 8), #5(w9), #8 (w5-9), #9 (w5, 6, 8-9), #11(w9), #14 (w8-9), #19 (w5-9) ↓ #3 (w5) **Stem diameter** ↑ #19, ↓ #14, #15, **Flowering time** ↓ #14, #15, #19 **Flower head diameter** ↑ #8, #9, #10, #17, #19 **Flower number** ↑#7, #15, #19 ↓ #17

CON = control, FER = Ferbon® (FF50SB, a lignite-based soil conditioner, 150, 300 and 450 kg/ha), LIF = liquid inorganic fertiliser (Hoagland's solution, 0, 50 and 100% strength of standard rate of final solution) and EM = effective microbes™ (Vital Resource Management Pty Ltd, 15 and 30 L/ha). PH = plant height, SD = stem diameter, FT = flower time, F# = flower number, FH = flower head diameter and w# = week after transplanting.

Table 7.2 Key findings of the trials presented in Chapter 4 for selected parameters. Treatments with significantly different values compared to other treatments are highlighted (↑ significantly increased, ↓ significantly decreased) and those not different are not shown.

Chapter	Treatments	Key findings
4 (sunflower trial 1 – a basic potting mix)	1. CON - AMF 2. CON + AMF 3. LOF (50%) - AMF 4. LOF (50%) + AMF 5. LIF (100%) - AMF 6. LIF (100%) + AMF 7. - AMF (main effect) 8. + AMF (main effect) 9. CON (fertiliser, main effect) 10. LOF (50%) main effect 11. LIF (100%) main effect	**Plant height** ↑ #6 (w5-9), #8 (w5-10), #11 (w5-10), ↓ #3 (w5-9), #10 (w5-9), **Stem diameter** ↑ #5, #11 and ↓ #8, #10, #3 **Flowering time** ↑ #10 and ↓ #11 **Flower head diameter** ↑ #8, # 11 **AMF colonisation** Hyphae = ↑ #8, #10 and ↓ #11 Vesicles = ↑ #10 and ↓ #11 Arbuscules = ↑ #8, #10 and ↓ #11
4 (sunflower trial 2 – a basic potting mix)	1. CON - AMF 2. CON + AMF 3. LOF - AMF 4. LOF (50%) - AMF 5. LOF (50%) + AMF 6. LOF (100%) - AMF 7. LOF (100%) + AMF 8. LIF (100%) - AMF 9. LIF (100%) + AMF 10. LOF (50%) + LIF (100%) - AMF 11. LOF (50%) + LIF (100%) + AMF 12. LOF (100%) + LIF (100%) - AMF 13. LOF (100%) + LIF (100%) + AMF 14. - AMF (main effect) 15. + AMF (main effect) 16. Control (fertiliser, main effect) 17. LOF (50%) main effect 18. LOF (100%) main effect 19. LIF (100%) main effect 20. LOF (50%) + LIF (100%) main effect 21. LOF (100%) + LIF (100%) main effect	**Plant height** ↑ #15 (w4, 5, 6, 7, 8) **Stem diameter** ↑ #19, **Flowering time** ↓ #15 **Flower head diameter** ↑ #19, # #20 **Flower number** ↑ #19, # #20 **AMF colonisation** Hyphae = ↑ #15 Vesicles = ↑ #15 Arbuscules = ↑ #2, #7, #11, #15, #17, #18 and ↓ #19 **Leaf nutrients** **Total N** = ↑ #18, #19 and #20; **P** = ↑ #7, #18 and #21 **K** = ↑ #11 and #20; **Ca** = ↑ #17 and #18; **B** = ↑ #11 and #15 **Mn** = ↓#15 and ↑#4, #17 and #18; **Zn** = ↑#17 and #18

CON = control, LOF = liquid organic fertiliser (a commercial product based on seaweed extracts 'QuadShot® (SLTEC), 0, 50 and 100% of strength of label recommendations), LIF = liquid inorganic fertiliser (Hoagland's solution, 0, 50 and 100% strength of standard rate of final solution) and EM = effective microbes™ (Vital Resource Management Pty Ltd, 15 and 30 L/ha). PH = plant height, SD = stem diameter, FT = flower time, F# = flower number, FH = flower head diameter and w# = week after transplanting.

Table 7.3 Key findings of the trials presented in Chapter 5 for selected parameters. Treatments with significantly different values compared to other treatments are highlighted (↑ significantly increased, ↓ significantly decreased) and those not different are not shown.

Chapter	Treatments	Key findings
5 (sunflower trial 1 – Forest soil (high nutrient))	1. CON - AMF 2. CON + AMF 3. FER - AMF 4. FER + AMF 5. COM - AMF 6. COM + AMF 7. SHG - AMF 8. SHG + AMF 9. COM+SHG - AMF 10. COM+SHG + AMF 11. - AMF (main effect) 12. + AMF (main effect) 13. CON (main effect) 14. FER (main effect) 15. COM (main effect) 16. SHG (main effect) 17. COM+SHG (main effect)	**Plant height** ↑ #15 (w7-10), and ↓ #16 (w7-10) **Stem diameter** ↑ #16 **Flowering time** ↑ #8 and ↓ #2 **Flower head diameter** ↑ #13, #15 and #17 **Flower number** ns **AMF colonisation** Hyphae = ↑ #12, #14, #15 and #16 Vesicles = ↑ #14 Arbuscules = ↑ #12,#14 and #15 **Leaf nutrients** **Total N =** ↑ #2, #3, #4, #5,#7, #9, #10; and ↓ #1, #6 and #8 **K =** ↑#12; **Mg =** ↓#12; **Zn =** ↓ #12 **Soil nutrients** **NH_4^+ - N =** ↑ #1, #7, #16 and ↓ #2, #6, #12, #15; **NO_3^- - N** = ↑ #7, #16 and ↓ #2, #6, #12, #15; **P =** ↑ #16 and ↓ #12, #15; **K =** ↑ #3, #7, #14, #16 and ↓ #2, #5, #9, #12, #15, #17; **Exc. Ca =** ↑ #4, #12, #14, and ↓ #7, #15, #16; **Exc. Mg** = ↑#3, #14, and ↓ #9, #17; **Exc. K =** ↑ #3, #7, #8, #14, #16 and ↓ #9, #15; **B** = ↑ #3, #7, #8, #10, #14, #16 and ↓ #6, #15; Fe = ↑ #15, #9, #16 and ↓ #10; **Mn =** ↑ #9, #14, #15, #16 and ↓#10, #12, **Zn =** ↑ #9, #15, #16 and ↓#10, #12; **pH =** ↑ #10, #12, #14, #17and ↓ #7, #16;
5 (sunflower trial 2 – Orchard soil (low nutrient)	1. CON - AMF 2. CON + AMF 3. FER - AMF 4. FER + AMF 5. COM - AMF 6. COM + AMF 7. SHG - AMF 8. SHG + AMF 9. COM+SHG - AMF 10. COM+SHG + AMF 11. - AMF (main effect)	**Plant height** ns **Stem diameter** ↑ #5 and #8; ↓ #7 and #10 **Flowering time** ns **Flower head diameter** ↑ #12, #14 and #16 **Flower number** ns **AMF colonisation** Hyphae = ↑#2, #6, #9, #10, #12, #15 and #17 Vesicles = ↑ #12 Arbuscules = ↑ #2, #6, #8, #9, #10, #12, #15 and #17

12. + AMF (main effect) 13. CON (main effect) 14. FER (main effect) 15. COM (main effect) 16. SHG (main effect) 17. COM+SHG (main effect)	**Leaf nutrients** **K =** ↑ #12, #14 and #17; **Ca =** ↑ #3, #4, #5, #9 and ↓#12; **B =** ↑ #14 **Mn =** ↑ #3, #4, #9, #14, #15, #17 and ↓#1, #8, #10; **Zn =** ↑ #3, #4, #5, #9, #14, #17 and ↓ #1, #6, #7, #10, #16 **Soil nutrients** **NH_4^+ - N =** ↑ #12 and ↓ #16; **NO_3^- - N** = ↑ #12, #14, #17 and ↓ #16; **P =** ↑ #2, #6, #10, #12, #15, #17 and ↓#4, #8 ; **K =** ↑ #6, #10, #12, #15, #17 and ↓#5, #16; **Exc. K =** ↑ #2, #6, #10, #12, #15, #17 and ↓ #7, #16; **Exc. Ca =** ↑ #6, #9, #12, #15 and ↓ #2, #14; **Exc. Mg =** ↑ #6, #12, #15 and ↓ #3, #14,; **Fe =** ↑ #5, #9, #15, #17 and ↓ #2, #12; **Mn =** ↑ #6, #15 and ↓ #2, #16; Zn = ↑ #4, #12, #14 and ↓ #2,#7, #16; **pH =** ↑ #7, #9, #15, #16, #17 and ↓ #8, #12, #14;

CON = control, FER = Ferbon® (FF50SB, a lignite-based soil conditioner, 150, 300 and 450 kg/ha), COM = compost (Foundation aerobic compost, Pure Living Soil Pty Ltd) and SHG = soluble humate granules (Nutri Tech Solutions®). PH = plant height, SD = stem diameter, FT = flower time, F# = flower number, FH = flower head diameter and w# = week after transplanting.

Table 7.4 Key findings of the trials presented in Chapter 6 for selected parameters. Treatments with significantly different values compared to other treatments are highlighted (↑ significantly increased, ↓ significantly decreased) and those not different are not shown.

Chapter	Treatments	Key findings
6 (apple trial – Royal Gala, sandy loam)	1. CONV 2. ALT 3. HERB 4. BC 5. CC 6. CONV + BC 7. ALT + BC	**AMF colonisation** **Hyphae =** Spring 2013 = ↑ #4; Autumn 2014 = ↑ #4 **Vesicles =** Spring 2013 = ↑ #4; Autumn 2014 = ↑ #4, #5; Spring = 2014 ↑ #4 **Arbuscules =** Spring 2013 = ↑ #1, #4; Spring = 2014 ↑ #4; **Total AMF =** Spring 2013 = ↑ #4; Spring = 2014 ↑ #4; **Fruit flavour characteristics** **TSS =** ↑ #1, #2, #3, #5 **TA =** ↑ #1, #2, #3, #5 **TSS/TA =** ↓ #1, #5
6 (cherry trial 1 – Lapin, loam)	1. CONV 2. ALT 3. - EM 4. + EM 5. CONV + EM 6. ALT + EM	**AMF colonisation** **Hyphae =** Spring 2013 = ↑ #3; Spring = 2014 ; **Vesicles =** Spring 2013 = ↑ #3; Summer 2014 = ↑ #1, #6; Spring = 2014 ↑ #2; **Arbuscules =** Spring 2013 = ↑ #3; Spring = 2014 ↑ #1, #6; **Total AMF =** Summer 2014 = ↑ #1, #6 **Fruit flavour characteristics** **TSS =** ↓ #1, #3 **TA =** ns **TSS/TA =** ns
6 (cherry trial 2 – Sweetheart, dolerite/clay)	1. CONV 2. ALT 3. - EM 4. + EM 5. CONV + EM 6. ALT + EM	**AMF colonisation** **Hyphae =** ns **Vesicles =** Spring 2013 = ↑ #3; Summer 2014 = ↑ #2, #5 **Arbuscules =** Summer 2014 = ↑ #4; Spring = 2014 ↑ #5; **Total AMF =** Spring 2013 = ↑ #4; Summer 2014 = ↑ #2, #5 **Fruit flavour characteristics** **TSS =** ↑ #1, #3 **TA =** ↑ #1 **TSS/TA =** ns

CONV = conventional nutrient regime, ALT = alternative nutrient regime, HERB = herbicide, BC = biochar, CC = cover crops (clover/grass) and EM = effective microbes™ (Vital Resource Management Pty Ltd, 15 and 30 L/ha). TSS = total soluble solids, TA = titratable acidity as malic acid and TSS/TA = the ratio of TSS to TA.

7.2 Impact of bio-fertilisers

Overall, soil inoculation with EM in conjunction with different nutrient regimes showed that EM improved sunflower shoot growth (Chapter 3), but EM had a mixed influence on AMF colonisation in cherry roots depending on the nutrient regime and soil type, with no effects on fruit flavour characteristics (Chapter 6).

Firstly, a key finding was that doubling the rate of EM reduced the beneficial effects of EM inoculation on sunflower growth (Chapter 3). This may be attributed to the microbes when applied at the higher rate utilising some of the nutrients, such as N, that would otherwise have been available for the plant. The negative effect of high EM concentrations was removed when combined with the full concentration of liquid inorganic fertiliser (LIF) which demonstrates the importance of ensuring sufficient nutrient availability when applying bio-fertilisers such as EM to the soil. This is supported by Yamada and Xu (2001) who reported that the success of EM inoculation to stimulate plant growth depends on the nutritional status of the soil. An imbalance between soil micro-organisms and available nutrients can also impact in other ways on plant growth. EM play an important role in nutrient recycling in the soil. Breitenbeck *et al.* (1980) reported that micro-organisms contribute significantly to the loss of N through the process of denitrification within 2 to 3 weeks of addition. Furthermore, soil micro-organisms produce secretions of organic materials that mimic the effect of plant hormones (Higa and Parr, 1994). Hence, stimulation of plant growth through the use of bio-fertilisers, combined with nutrient supplements, may not be due solely to an increase in available nutrients, but may, in part, be due to an increase in microbial activity.

The observed reduction in leaf chlorophyll content of sunflower following EM inoculation in this study (Chapter 3) may be attributed to the increased biomass observed in EM treated plants diluting N levels and consequently chlorophyll content. Cechin and de Fátima Fumis (2004) reported that up to 75% of leaf-N exists in the chloroplasts, and they attribute low levels of chlorophyll to declining photosynthetic processes under conditions of limited N. Additionally, according to Evans *et al.* (2000), most of the organic N in protein form can be distributed in different parts of the plant in order to increase photosynthesis, and this deployment could be related to the content of leaf chlorophyll. Ingestad (1979) also concluded that distribution of nutrients can occur between leaves of different ages, leading to competition between leaves impacting on chlorophyll content.

The effect of soil inoculation by EM on fruit flavour characteristics observed in this study has demonstrated some unexpected impacts of bio-inoculants. The observed improvement in TSS content in both ‘Lapin’ and ‘Sweetheart’ cherry fruit in trees not receiving EM application (Chapter 6) may be linked to a decline in AMF colonisation following EM inoculation. Many studies discuss the role of AMF in enhancing uptake of many nutrients (Hattingh *et al.*, 1973, Ames *et al.*, 1983, George *et al.*, 1992, Smith *et al.*, 2000, Wang *et al.*, 2002). In the studies reported in this dissertation, EM inoculation reduced AMF colonisation and TSS content was

reduced, hence it could be argued that in the absence of EM inoculation, AMF colonisation and consequently nutrient uptake are likely to improve and hence fruit TSS content may be enhanced. On the other hand, it is also possible that soil K availability impacted fruit quality. In both the conventional and alternative treatments, total K was 50% higher than the baseline and 32% higher than in the corresponding EM treatments; a similar pattern was observed with exchangeable K levels. Hence, available K was lower in EM inoculated soil, and as an increase in absorption of K can increase fruit TSS content this most likely contributed to the observed differences between inoculated and non-inoculated trees. Nava *et al.* (2007) reported that K deficiency prevents the biosynthesis of organic acids, sugars and vitamin C, resulting in lower fruit TSS content.

AMF inoculation improved apical sunflower growth in the presence of an adequate amount of nutrients (LIF) compared to plants receiving AMF only (Chapter 4). The observed increase in plant height with AMF along with nutrient application appears to be largely influenced by nutrient concentration, particularly N. The amount of N added weekly in the inorganic fertiliser (LIF) treatments was several times greater than the N added in the organic fertiliser (LOF) treatments. Massey (1971) observed that sunflower height increased 11 cm compared to the control when plants were fertilised with 56 kg N per hectare, while stem diameter was significantly influenced by plant spacing instead of N fertiliser. Ozer *et al.* (2004) and Ayub *et al.* (1998) noticed that different rates of N increased stem height and diameter of sunflower. As N is a key player in the growth and development of plant biomass it is likely that the application of nutrients enhanced the abundance of N, therefore its absorption was improved.

Results also suggest AMF inoculation increased sunflower shoot growth and flower head diameter, possibly through increasing uptake of other nutrients such as B, as seen by an increase in leaf B concentrations (Chapter 4). Asad *et al.* (2003) and Asad (2002) suggested that sunflower is highly sensitive to low B supply, with deficiencies reducing vegetative growth and the development of reproductive organs. McIlrath and Skok (1964) also reported that sunflower height and internode length significantly increased in plants that received B nutrition compared to plants that received no B. Farokhi *et al.* (2014) found that B nutrition increased the flower head size of sunflower plants. Our findings should encourage broader research as to whether B was responsible for improving plant apical growth under different levels of B concentrations when other factors, including activation of soil microbes (soil fumigation), soil fertility and nutrient availability, are controlled.

In contrast to EM inoculation, results showed that AMF inoculation improved AMF colonisation structures in both trials. AMF inoculation improved leaf chlorophyll content when adequate N was provided (Chapter 4). This is supported by the increase in leaf chlorophyll observed in the inorganic fertiliser treatments, which contained large amounts of N. Leaf chlorophyll content was also lower in the AMF and organic fertiliser combination or control plus AMF compared with inorganic fertiliser in the first 6 weeks from transplanting. This can be attributed to higher amounts of N added by inorganic application in comparison with the quantities added by the organic application as discussed above.

In the presence of adequate levels of N, both EM and AMF have the ability to enhance leaf chlorophyll content. However, in N limited conditions, the high rates of EM can lead to a decrease in leaf chlorophyll content as a result of competition for N (Yamada and Xu, 2001), or due to the loss of N from the soil by denitrification (Breitenbeck *et al.*, 1980). Likewise, under N limited conditions, results suggest it is better not to have AMF colonisation, as the AMF may decrease leaf chlorophyll content as a result of competition for N. Hodge and Storer (2015) reported that under low N conditions, competition for available N between the plant and fungal symbionts can be increased. This was observed in this study with a slight decrease in leaf chlorophyll content in AMF inoculated plants compared to uninoculated plants (Chapter 4 - Trial 1).

The importance of EM inoculation for non-leguminous crops such as sunflower in improving leaf chlorophyll content may be manifested by facilitating or fixing nutrients in the soil, especially those that enter or contribute to the synthesis of chlorophyll such N and P. Alongi (1994) showed that microbes are efficient recyclers of soil nutrients, but this efficiency varies depending on the prevailing environmental conditions. The situation may be different for AMF inoculation; AMF may enhance leaf chlorophyll content by improving P uptake, especially at low levels of N, which plays an essential role in the synthesis of chlorophyll. This is supported by the increase in leaf chlorophyll content when plants were inoculated with AMF and received the 50% rate of liquid organic fertiliser (LOF). Liquid organic fertiliser (50% label rate) in the first trial increased AMF colonisation (Chapter 4 - Trial 1), which may have led to a significant increase in the P content of the leaf, given that combined treatment of AMF and LOF (100% label rate) in Trial 2 increased leaf-P concentrations. Plesničar *et al.* (1994) observed a decrease in the leaf chlorophyll content in sunflower plants under P-deficiency. Consequently, in addition to N effects, EM and AMF can be one of the

factors influencing the leaf chlorophyll content when they are able to improve the readiness or the absorption of P.

In summary, inoculation with bio-stimulants such as EM or AMF can have beneficial effects on sunflower growth in the presence of sufficient amounts of nutrients, particularly N. When applying EM it is important to ensure that sufficient N is available in order to avoid the negative effects of N-deficiency due to competition (between EM and plants) or loss from the soil (denitrification process).

7.3 Effect of fertiliser type

Many inorganic nutrients used as fertilisers are often in concentrated forms readily available to plants, but these nutrients are often quickly lost from the soil, and can cause environmental problems (Reganold *et al.*, 1993, Welbaum *et al.*, 2004). While most nutrients in solid organic supplements need to be converted to inorganic forms by soil microorganisms to become available for absorption by plants, they are also released slowly (Baziramakenga and Simard, 2001, Mkhabela and Warman, 2005, Risse and Faucette, 2009, Baldi *et al.*, 2010). As such, we explored the recent trend of liquid organic fertilisers containing nutrients in a more plant available form.

7.3.1 Leaf nutrients relevant to fertiliser type

In comparing commercial liquid organic fertiliser with liquid inorganic fertiliser in the form of Hoagland's solution, the studies show that organic fertiliser based on seaweed extracts (full rate) can improve sunflower foliar nutrition, particularly P and Mn. Trial results showed that foliar K and B increased when organic fertiliser (half rate) was applied in combination with inorganic fertiliser plus AMF (Chapter 4 - Trial 2). The liquid organic fertiliser used in these studies contained a higher concentration of P and an equivalent level of K to the inorganic fertiliser. As explained previously, LOF had negligible impact on the growth of sunflower. Therefore, the increase in the concentrations of nutrients, especially P and K may be due to the higher supply of these nutrients compared with the size of the biomass produced by organic fertiliser.

The presence of additional compounds in the organic fertiliser may also have contributed to the increase in leaf nutrients through improved nutrient uptake. Crouch *et al.* (1990) suggest there is a belief that applications of seaweed extracts like LOF may contain organic molecules that act as a chelate for some nutrients in the soil, thereby increasing their bioavailability. Moreover, humic acid, another component of the organic fertiliser, acts in a

similar way as plant hormones (Chen and Aviad, 1990, Nardi *et al.*, 2002, Thirumaran *et al.*, 2009, Ouni *et al.*, 2014). Seaweed extract organic fertilisers also contain plant hormones including cytokinins (Durand *et al.*, 2003, Stirk *et al.*, 2003), auxins (Stirk *et al.*, 2004), gibberellins and betaines (Kumar and Sahoo, 2011). There is increasing evidence that nutrient absorption and mobility within the plant is under the control of hormones (Krouk *et al.*, 2011). Hence these organic compounds are likely to have contributed to the increase in the absorption of nutrients from the organic fertiliser treated plants. The use of organic nutrients in liquid form can provide necessary nutrients for growth of annual crops in a short time, thus avoiding deficiencies that may result from the slow release of nutrients from solid organic matter, especially in short term cropping.

7.3.2 Soil nutrients relevant to fertiliser type

Many studies have reported the success of solid organic supplements in compost form as it can modify soil properties or act as a source of mineral nutrients. Trials in this study show that the use of humic supplements such as Ferbon® and soluble humate granules (SHG) as a new approach can achieve the same goals of using compost and may even be better in some cases. Results from these trials have shown that humates facilitated greater release of nutrients in the soil compared to compost (Chapter 5). In the pot trials, SHG increased the availability of most nutrients in the orchard soil, but in the forest soil compost improved the release of nutrients. The availability of mineral nutrients in the soil was significantly affected by soil pH (Lyle *et al.*, 2006). In the orchard soil, soluble humate granules (SHG) and compost together caused a decrease soil pH from 5.9 to 5.6 and 5.9 to 5.5 respectively, which may have played an important role in the release of nutrients. Although SHG and compost also reduced the pH of the forest soil from 5.7 to 4.6, this may be too low for the release of most of nutrients. Therefore, the increase in the release of nutrients in the forest soil may be due to other factors such as C:N or C:P ratio which were not considered in this trial.

Plaza *et al.* (2006) and Xiong *et al.* (2010) show that the significant influence of humic substances on the facilitation of mineral nutrient release, especially heavy mineral nutrients such as Zn, is due to the high molecular weight of these humic substances (e.g. humic and fulvic acid) with low acidic functional groups. Humic substances increase or decrease the release of nutrients in the soil through the formation of complex compounds, either soluble or insoluble, with metal ions; this is dependent on soil pH. Consequently, humic substances can play a dual role in the soil. In the current trials, the orchard soil pH may be at the appropriate level for the formation of soluble complex compounds with humic substances, while the

acidity of the forest soil may have enhanced the formation of insoluble complex compounds with some nutrients.

In the commercial orchard apple site, results showed that both nutrient regimes (alternative and conventional) improved the abundance of nutrients in the soil compared to the baseline levels prior to trial commencement (Chapter 6). Although the humate amendment (Ferbon®) was blended with targeted minerals, the chemical analysis of the soil did not unequivocally confirm any substantial increases in total and available nutrients in the soil compared with the conventional nutrient regime. This may be due to the higher electrical conductivity (EC) observed in the alternative treatment regime. High EC may lead to salinisation of the soil and N depletion and can reduce the cycling of nutrients (Stamatiadis *et al.*, 1999).

In the cherry trial sites, blended humate additions under the alternative nutrient regime also improved total nutrient content and the availability of some nutrients. Mg salts decreased with the addition of humate, while Ca salts slightly increased. Arshad and Coen (1992) showed that the ratio of Na^+ to Ca^{2+} plus Mg^{2+} ion concentrations affect EC value and Bruce *et al.* (1988) found that $CaSO_4$ increased EC value. Additionally, it is well known that soil pH plays a major role in the absorption of nutrients (Hyland *et al.*, 2005, Lyle *et al.*, 2006). Therefore, the decline in soil pH in the Lapin sweet cherry orchard may have been a major factor affecting the availability of some nutrients.

In pot trials (Chapter 5), results showed that SHG was a better amendment to improve nutrient release in the soil compared to Ferbon. In both the cherry and the apple trials, Ferbon® (FF50SB) was used as the humate addition, which was blended with fish oil according to the product label. Molecules of humic compounds in Ferbon may have formed complex compounds with the fish oil, which can make them unable to change the chemical properties of the soil until the fish oil decomposes into simple organic compounds. The increase in leaf chlorophyll content observed in Trial 2 (Chapter 3) and Trial 5 (Chapter 5) supports this idea. Leaf chlorophyll content improved in week 8 for plants receiving 450 kg ha^{-1} Ferbon and 15 L ha^{-1} EM, while improvement in leaf chlorophyll content was observed in week 5 when plants were fertilised with 450 kg ha^{-1} Ferbon plus the double rate of EM (30 L ha^{-1}). Similar results were observed in pots amended with 300 kg ha^{-1} Ferbon after 7 weeks of transplanting, whereas leaf chlorophyll content and foliar nutrient levels improved in the same treatment including K.

7.4 Effects on AMF colonisation

The liquid organic fertiliser showed stimulatory effects on root colonisation by AMF, whilst the formation of arbuscules substantially improved in inoculated plants (Chapter 4). Humic substances have been shown to improve AMF colonisation. For example, Gryndler *et al.* (2005) observed that humic and fulvic acids stimulated AMF colonisation on maize roots in a hydroponic culture, and further suggested that humic substances may also stimulate AMF colonisation in the soil environment.

AMF colonisation was not detected in inoculated plants receiving LIF (Chapter 4 - Trial 2). High concentrations of P are known to lead to low AMF colonisation (Verkade and Hamilton, 1983, Koide, 1985, Thompson, 1987, Johnson, 2010). Although LIF contained low P-levels compared to LOF, it had high levels of N which may have played a role in the reduction of AMF colonisation. Fertilisers rich in N can indirectly affect the mutual benefits and the formation of the symbiotic associations between the host plant and AMF. Mäder *et al.* (2000) reported that intensive agriculture or nutrient enrichment by N and P inputs reduces the benefits of the mycorrhizal symbiosis. Mäder *et al.* (2000) demonstrated that host plants exposed to high nutrient levels severely reduced or stopped providing their fungal partners with carbohydrate sources that they need and in turn reduced AMF colonisation. Therefore, the reduction in AMF colonisation in LIF plants may be due to the abundance of nutrients such as N, which made the symbiotic associations with AMF irrelevant. It can be concluded that, in the presence of limited levels of P, plant performance was better with LIF, although mycorrhizal colonisation increased with LOF.

Addition of compost to plants inoculated with AMF increased hyphal colonisation, while numbers of arbuscules responded differently to all organic supplements (Chapter 5). The differing effects of these organic supplements between soil types are likely to be due to the difference in fertility of soils in which the plants were grown. The increase in hyphal colonisation when compost was applied may be a result of the lower P concentrations in the compost at a level that did not inhibit colonisation. This is supported by Vaidya *et al.* (2008) who reported that AMF spore density tends to increase in soils treated with compost compared with those receiving inorganic P. Compost often also increases both hyphae and arbuscules because it may bring with it AMF propagules (Cavagnaro, 2014, Cavagnaro, 2015). Results also showed that a combination treatment of AMF inoculation and SHG improved the number of arbuscules. As the effects of organic supplements, including SHG, on AMF colonisation were examined in two different soil types, it is likely that effects of

SHG in the orchard soil were associated with nutrient concentrations, including P, which were having the greatest influence on AMF colonisation.

In the apple orchard site, results show that the formation of symbiotic associations between apple roots and AMF is always controlled by nutrient levels, like that of P. Thus, it was hypothesised that orchard floor management can be manipulated to enhance these associations. For example, the addition of biochar, as well as treatment with cover crops in some cases appeared to result in positive effects upon AMF colonisation. Firstly, in the case of biochar, changes in AMF colonisation did not appear to be due to changes in the chemical characteristics of the soil, such as P content, but, rather, may have resulted from changes in the microbial biomass in the soil. Warnock *et al.* (2007) reported that biochar additions may lead to changes, either beneficial or harmful, in other soil microbes, such as phosphate solubilizing bacteria (PBS) or mycorrhization helper bacteria (MHB). These authors further proposed that biochar acts as a refuge for hyphal grazers. Secondly, with regard to treatment with cover crops, P-concentration in the soil may fall due to P uptake by cover crops. Cover crops have been shown to enhance AMF colonisation by increasing P uptake (Kabir and Koide, 2000); this works by reducing the negative effects of high concentrations of P on AMF colonisation. In addition, the roots of the cover crops can maintain an appropriate network of mycorrhizal hyphae and spores (Dodd and Jeffries, 1986, Dabney *et al.*, 2001). Hence, it appears that cover crops provide a source of AMF inoculum, perhaps in the same way that the cover crops in the present study supplied the apple roots with a source of AMF colonisation.

In addition to the impact of horticultural practices such as nutrient management, orchard floor management and bio-fertiliser inoculation, results showed the strong influence of season on total AMF colonisation and root richness with vesicles. The number of vesicles was increased during autumn 2014 in ‘Royal Gala’ apple roots and summer 2014 in both ‘Lapin’ and ‘Sweetheart’ cherry roots (Chapter 6). This may be due to seasonal constraints in AMF vesicles as a consequence of physiological and adaptive patterns of the species applied (Purin *et al.*, 2006). The observed decline in AMF vesicles in the first season might be a reaction to practices utilised such as nutrient application and/or orchard floor management by the grower. Another factor influencing the percentage of AMF colonisation and high formation of vesicles in the cherry roots may be the physiological peak of the trees during the summer season where greater root carbohydrate exudates are likely to be available. Purin *et al.* (2006) reported a similar finding for apple roots.

7.5 Fruit flavour response

All three orchard trials showed positive correlations between AMF colonisation and flavour characteristics of both apple and sweet cherry fruit (Chapter 6). The intensity of these correlations was associated with the type of nutrient regime used and the orchard floor management strategy applied at the site.

Both TSS content and MA level were associated with the degree of total AMF colonisation in the fruit. The alternative nutrient regime improved TSS content, but MA levels were higher in the conventional regime. Application of inorganic fertilisers in the conventional regime provided nutrients in an available form which can be acquired quickly by plant roots, in addition to the contribution of AMF in acquiring nutrients; high nutrient levels, particularly N, can be associated negatively with fruit characteristics. The slow liberation of nutrients including N and K from organic nutrient sources with AMF contribution can be in the optimal level to improve fruit flavour characteristics. Reig *et al.* (2006) state that the supply of N from organic fertilisers is more balanced (gradual release nutrients) compared with N added by conventional fertilisers. The improvement in TSS content, which was offset by a decrease in the MA level, may be due to lower N and higher K absorption in the alternative nutrient regime, in contrast to a higher N with the conventional nutrient regime. The importance of N and K nutrition for increasing or decreasing TSS content and MA level has been observed in other studies. Nava *et al.* (2007) found that TSS content in apple fruit was negatively correlated with N fertilisation, while positively influenced by K fertilisation. Reig *et al.* (2006) reported that organic fruit contained lower NO_3^- concentrations. The latter can be explained by a balanced supply of nutrients in organically managed soils compared to conventional production methods based on high N inputs. This is also evidence that supports improved TSS content under the alternative regime. Soil chemical analysis showed lower K content in the alternative regime compared to conventional. Further, a decline in soil content of K may be due to uptake by the tree or leaching with irrigation water. If K is absorbed by the trees, that means that the results of this trial are consistent with the findings of Nava *et al.* (2007).

The increase in MA level observed in the conventional nutrient regime could also be closely dependent on ready availability and high uptake of other nutrients. In addition to N, soil P and Mg were reasonably influenced by the conventional regime. As high nutrient concentrations can positively associate with fruit flavour characteristics, this may be another reason for the high MA level in the conventional treatment. The study by Casero *et al.*

(2004), who found that fruit acidity of 'Golden Smoothee' apples was positively correlated with P, K, Mg and Ca nutrients in both the fruit and leaf, confirms this. Regardless of how N is added to the soil, it can be concluded that its increase in the soil can lead to increased MA level in apple fruits.

This study has also shown that orchard floor management can have an impact on fruit characteristics (Chapter 6). The level of MA was higher in apple fruit from trees with in-row grass/clover swards. This may be due to the high N-concentrations delivered to fruits by trees. It is well known that many cover crops, especially legumes, have the ability to fix atmospheric N, which means more available N in the soil (Dabney *et al.*, 2001). Additionally, Hoagland *et al.* (2008) noticed that weed control by tillage led to satisfactory growth of apple trees, as a result of desirable levels of N and most other nutrients. In the current trial, control of weeds using herbicides may have resulted in the same results found by Hoagland. The negative association of TSS/TA ratio with AMF colonisation in the conventional regime and cover crop floor management may be due to the improvement of TSS instead of MA for the reasons explained above, as both treatments positively improved TSS content.

7.6 General Conclusion

The research presented here has shown that using humic supplements such as SHG and Ferbon® can result in similar crop productivity that can be obtained from compost in improving soil fertility. The results also show that SHG has the potential to improve the liberation of most nutrients in the soil, while compost remains better in promoting AMF colonisation followed by Ferbon. Additionally, a combination of bio-inoculants and renewable alternative nutrient regime can lead to the same result as conventional nutrient regimes, especially when combined with N additions. Dissoluble and granular organic supplements can be a good amendment to the soil, especially as they are easy to apply compared to compost. On the other hand, in comparison with AMF colonisation in the conventional nutrient regime, it has been shown that AMF colonisation along with the alternative nutrient regime applied here can improve fruit flavour characteristics such as total soluble solids (TSS) and malic acid (MA) in apple fruit. In contrast, for cherry roots, the effects of AMF colonisation on TSS content and MA level were better with the conventional nutrient regime.

Positive effects, or those barely noticeable, of organic practices on growth attributes and yield quality characteristics raises many questions about finding proper approaches to the use of

humic substances, the type of nutrient regimes and orchard floor managements to achieve agricultural sustainability. These questions cannot be answered by limited studies; therefore, additional research is required.

7.7 Further future research

1. Research here examined both EM and AMF as independent biological treatments but did not assess them as a mixed treatment. Future studies could investigate their interaction.
2. Currently there are few studies which show that the type of nutrients used to enrich the soil (conventional inorganic fertiliser applications) can lead to an imbalance in the microbial biomass as a result of increasing the concentration of nutrients in the rhizosphere. Previous studies indicated that the changes in N, P, S and Zn concentrations in the soil can negatively or positively affect AMF colonisation (Johnson, 2010, Johnson *et al.*, 2010, Kangwankraiphaisan *et al.*, 2013, Karaca, 2014, Ansori and Gholami, 2015). Whether foliar applications of nutrients lead to the same result has been little studied.
3. Seaweed extracts are frequently used as a renewable organic fertiliser instead of conventional fertiliser to nourish plants and enrich the soil with nutrients (Kumar and Sahoo, 2011). However, more research is required to examine the ability of these extracts to provide plants with sufficient amounts of nutrients, especially nitrogen, as in our studies we found that liquid organic fertiliser based on seaweed extracts had negligible influence on leaf chlorophyll content and increased leaf Ca, Mn and Zn but not total N that forms 75% of chlorophyll. Cechin and de Fátima Fumis (2004) observed that up to 75% of leaf-N exists in the chloroplasts in protein form; the researchers interpreted the decline of chlorophyll could be due to the decline in photosynthesis under nitrogen-limited conditions.
4. There are many organic materials such as compost, animal and plant waste used as supplements or organic soil amendments. Today there is a tendency to use humic compounds for the same purpose, but there is a belief that these humic substances have similar effects to plant hormones or act to regulate plant enzymes and hormones (Pettit, 2013, Zandonadi *et al.*, 2013). Therefore, further evaluation of the effects of these substances as foliar applications on the growth and yield of crops is required to refute or confirm this belief. However, it should be noted that there are limits as to how much nutrient can be applied to foliage before phytotoxicity is induced.

5. The present study demonstrated the effectiveness of humic compounds to improve soil fertility through facilitating the availability of nutrients in the soil. However, more attention is needed to understand the effects of humic compounds to facilitate mineral nutrients under different field conditions.
6. The present study demonstrated that mycorrhizal colonisation can positively influence flavour characteristics of perennial crop fruit. These results could be re-evaluated on fruit flavour of annual crops, to assess the impact of AMF in the short term compared to perennial crops. Furthermore, consumer testing under controlled trials could be used to evaluate the empirical results observed for TSS and MA.

In conclusion, this dissertation has sought to assess the effects of bio-fertilisers such EM and AMF, either in the short term (greenhouse trials) or long term (field trials) on mycorrhizal colonisation, nutrient status of the plant and the soil, growth and development of the plant and fruit quality. Also it sought to understand the effects of organic soil amendments, orchard floor managements and applications of different nutrients regimes on the same parameters above. This research will contribute to the development of integrated nutritional regimes, and has highlighted the importance of the interaction between mycorrhizal colonisation and nutrient management on the quality of the fruit flavour characteristics, and demonstrated that liquid organic fertilisers, particularly those based on seaweed extracts, cannot be solely relied upon for a sustainable soil fertility regime.

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"Chapter 9" Appendices

9.1 Appendix 1: Chapter 4

Table 9.1 Effects of interaction between mycorrhizal application (AMF) and fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on foliar macro and micro-nutrients of sunflower plant (Helianthus annuus L., 'Dwarf Sunsation').

Interactions		Total N (g/kg)	P (g/kg)	K (g/kg)	B mg/kg
Nil	Minus AMF	20.5	3.4 de	28.0 bcd	95.1 b
LOF (50%)		19.3	4.8 bc	28.3 bcd	96.7 b
LOF (100%)		19.0	4.6 c	29.0 bc	79.3 d
LIF (100%)		20.1	3.3 e	22.6 e	80.5 d
LOF (50%) + LIF (100%)		22.0	4.9 bc	29.8 b	73.3 d
LOF (100%) + LIF (100%)		18.5	5.3 b	26.3 cd	83.0 cd
Nil	Plus AMF	20.2	3.4 de	25.5 de	94.1 bc
LOF (50%)		18.0	4.5 c	25.1 de	80.6 d
LOF (100%)		21.9	6.0 a	27.4 bcd	92.6 bc
LIF (100%)		20.9	3.6 de	26.0 cd	83.1 cd
LOF (50%) + LIF (100%)		22.1	3.8 d	34.4 a	110.4 a
LOF (100%) + LIF (100%)		19.3	4.8 c	28.2 bcd	76.7 d
L.S.D.		*ns*	*1.01*	*3.30*	*11.89*
F prob.		*0.43*	*0.04*	*0.007*	*<0.001*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 9.2 Effects of interaction between mycorrhizal application (AMF) and fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) on foliar macro and micro-nutrients of sunflower plant (*Helianthus annuus* L., 'Dwarf Sunsation').

Interactions		Mn mg/kg	Zn mg/kg	Ca (g/kg)	Mg (g/kg)
Nil	Minus AMF	109.2 cd	72.02	47.7	10.5
LOF (50%)		139.0 b	90.26	43.6	9.3
LOF (100%)		170.9 a	90.48	42.9	8.3
LIF (100%)		100.0 cd	76.69	35.5	9.5
LOF (50%) + LIF (100%)		102.9 cd	77.60	37.6	9.3
LOF (100%) + LIF (100%)		110.9 c	73.95	43.5	8.7
Nil	Plus AMF	101.9 cd	79.57	47.3	9.2
LOF (50%)		102.7 cd	86.01	45.9	9.5
LOF (100%)		142.3 b	99.49	49.3	10.2
LIF (100%)		96.2 de	82.20	39.2	10.6
LOF (50%) + LIF (100%)		79.1 f	77.08	45.1	8.8
LOF (100%) + LIF (100%)		85.0 ef	75.75	40.9	8.9
L.S.D.		*14.48*	*ns*	*ns*	*ns*
F prob.		*0.01*	*0.59*	*0.36*	*0.57*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

Table 9.3 Effects of (1) mycorrhizal application (AMF), (2) fertiliser type [LOF (liquid organic fertiliser) and LIF (liquid inorganic fertiliser)) and (3) interaction between AMF and fertiliser type on foliar macro and micro-nutrients of sunflower plant (Helianthus annuus L., 'Dwarf Sunsation').

Treatments	Total N (g/kg)	P (g/kg)	K (g/kg)	B mg/kg	Mn mg/kg	Zn mg/kg	Ca (g/kg)	Mg (g/kg)
- AMF	19.9	0.44	27.3	84.6	122.1	80.2	41.8	9.2
+ AMF	20.4	0.43	27.8	89.6	101.2	83.4	44.6	9.5
L.S.D.	*ns*	*ns*	*ns*	*4.853*	*5.913*	*ns*	*ns*	*ns*
F prob.	*0.37*	*0.79*	*0.69*	*0.04*	*<0.001*	*0.19*	*0.06*	*0.35*
Nil	20.4 ab	3.4 c	26.7 b	94.6	105.5	75.79 b	47.5 a	9.8
LOF (50%)	18.7 b	4.7 ab	26.7 b	88.6	120.8	88.13 a	44.7 ab	9.4
LOF (100%)	20.4 ab	5.3 a	28.2 b	85.9	156.6	94.98 a	46.1 ab	9.2
LIF (100%)	20.5 ab	3.4 c	24.3 c	81.8	98.1	79.44 b	37.4 c	10.0
LOF (50%) + LIF (100%)	22.1 a	4.4 ab	32.1 a	91.8	91.0	77.34 b	41.3 bc	9.0
LOF (100%) + LIF (100%)	18.9 b	5.0 ab	27.2 b	79.8	98.0	74.85 b	42.2 bc	8.8
L.S.D.	*2.11*	*0.69*	*2.33*	*8.405*	*10.24*	*8.48*	*5.28*	*ns*
F prob.	*0.04*	*<0.001*	*<0.001*	*0.007*	*<0.001*	*<0.001*	*0.004*	*0.15*

Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level.

9.2 Appendix 2:

9.2.1 Interaction effects of AMF colonisation

Table 9.4 Mean presence of AMF colonisation structures in apple roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) orchard floor management (HERB, CC and BIO).

Nutrient regime	Orchard floor mgt.	Spring - 2013							
		H	H LOG(x+1)	V	V LOG(x+1)	A	A LOG(x+1)	AMF	AMF LOG(x+1)
	HERB	16.3	1.2	8.7	0.9	6.8	0.8	16.8	1.2
CONV	BIO	49.0	1.7	28.7	1.4	25.9	1.3	49.0	1.7
	CC	24.2	1.3	10.7	1.0	17.2	1.1	24.2	1.3
	HERB	19.5	1.3	7.0	0.9	9.3	1.0	19.5	1.3
ALT	BIO	28.7	1.4	17.7	1.2	12.2	1.1	29.0	1.4
	CC	21.5	1.3	8.3	1.0	6.8	0.9	21.5	1.3
LSD		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
F prob.		*0.10*	*0.16*	*0.36*	*0.50*	*0.10*	*009*	*0.11*	*0.18*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant, nd = not detected. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 9.5 Mean presence of AMF colonisation structures in apple roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) orchard floor management (HERB, CC and BIO).

Nutrient regime	Orchard floor mgt.	Autumn - 2014				Spring - 2014			
		H	V	A	AMF	H	V	A	AMF
	HERB	19.4	48.2	nd	61.0	60.0	34.4	3.8	61.0
CONV	BIO	40.6	57.9	nd	66.2	59.0	65.4	7.5	76.9
	CC	18.1	54.7	nd	62.3	46.0	44.2	0.6	59.4
	HERB	18.7	44.5	nd	59.2	34.0	45.2	1.9	52.9
ALT	BIO	24.7	58.0	nd	53.7	46.9	55.2	5.8	70.6
	CC	23.4	50.5	nd	50.7	51.9	37.9	0.8	57.5
LSD		*ns*	*ns*	--	*ns*	*ns*	*ns*	*ns*	*ns*
F prob.		*0.08*	*0.85*	--	*0.78*	*0.25*	*0.14*	*0.80*	*0.85*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant, nd = not detected. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 9.6 Mean presence of AMF colonisation structures in 'Lapin' cherry roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (minus and plus).

Nutrient regime	EM inoculation	Spring - 2013				Summer - 2014			
		H	V	A	AMF	H	V	A	AMF
CONV	- EM	53.5	23.0	18.9	58.5	39.8	48.5 a	10.9	61.9 a
	+ EM	26.5	9.3	4.1	26.7	32.9	30.2 bc	5.0	40.2 b
ALT	- EM	30.4	16.6	9.6	30.0	17.1	24.6 c	2.1	28.3 c
	+ EM	26.7	14.8	3.7	26.9	28.1	38.5 ab	3.3	46.7 b
LSD		*ns*	*ns*	*ns*	*ns*	*ns*	*12.96*	*ns*	*11.09*
F prob.		*0.22*	*0.12*	*0.16*	*0.16*	*0.22*	*0.002*	*0.22*	*0.004*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 9.7 Mean presence of AMF colonisation structures in 'Lapin' cherry roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (minus and plus).

Nutrient regime	EM inoculation	Spring - 2014			
		H	V	A	AMF
CONV	- EM	67.3	46.7	14.8 ab	70.6
	+ EM	44.4	31.0	7.5 b	49.0
ALT	- EM	58.1	53.8	11.3 ab	66.9
	+ EM	61.3	50.4	18.1 a	67.3
LSD		*ns*	*ns*	*7.52*	*ns*
F prob.		*0.07*	*0.38*	*0.01*	*0.11*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 9.8 Mean presence of AMF colonisation structures in 'Sweetheart' cherry roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (minus and plus).

Nutrient regime	EM inoculation	Spring - 2013				Summer - 2014			
		H	V	A	AMF	H	V	A	AMF
CONV	- EM	53.2	26.4	0.5	43.8	36.0	29.4 c	5.6	45.4 c
	+ EM	50.1	13.1	8.3	41.3	54.0	58.1 a	13.8	74.6 a
ALT	- EM	66.0	20.7	4.8	55.0	60.4	53.5 ab	6.9	71.9 ab
	+ EM	58.3	10.8	9.2	60.5	42.5	36.9 bc	17.3	56.5 bc
LSD		*ns*	*ns*	*ns*	*ns*	*ns*	*15.0*	*ns*	*15.67*
F prob.		*0.74*	*0.68*	*0.40*	*0.70*	*0.18*	*<0.001*	*0.85*	*0.001*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

Table 9.9 Mean presence of AMF colonisation structures in 'Sweetheart' cherry roots at different seasons of the year, subject to interaction effects between (i) nutrient regime (ALT and CONV) and (ii) effective microbes (minus and plus).

Nutrient regime	EM inoculation	Spring - 2014			
		H	V	A	AMF
CONV	- EM	33.8	21.5	2.7 b	34.2
	+ EM	61.3	35.9	16.3 a	45.6
ALT	- EM	45.4	26.9	5.4 b	61.7
	+ EM	36.9	20.9	6.1 b	37.1
LSD		*ns*	*ns*	*8.64*	*ns*
F prob.		*0.07*	*0.19*	*0.04*	*0.08*

*Means followed by the same letter within the same column and main effect are not significantly different at the 0.05 probability level, ns = not significant. H= hyphae, V = vesicles, A= arbuscules, AMF = AMF colonisation (presence).

9.2.2 Orchard maps

Figure 9.1 Nichols Rivulet (Huon Park) map

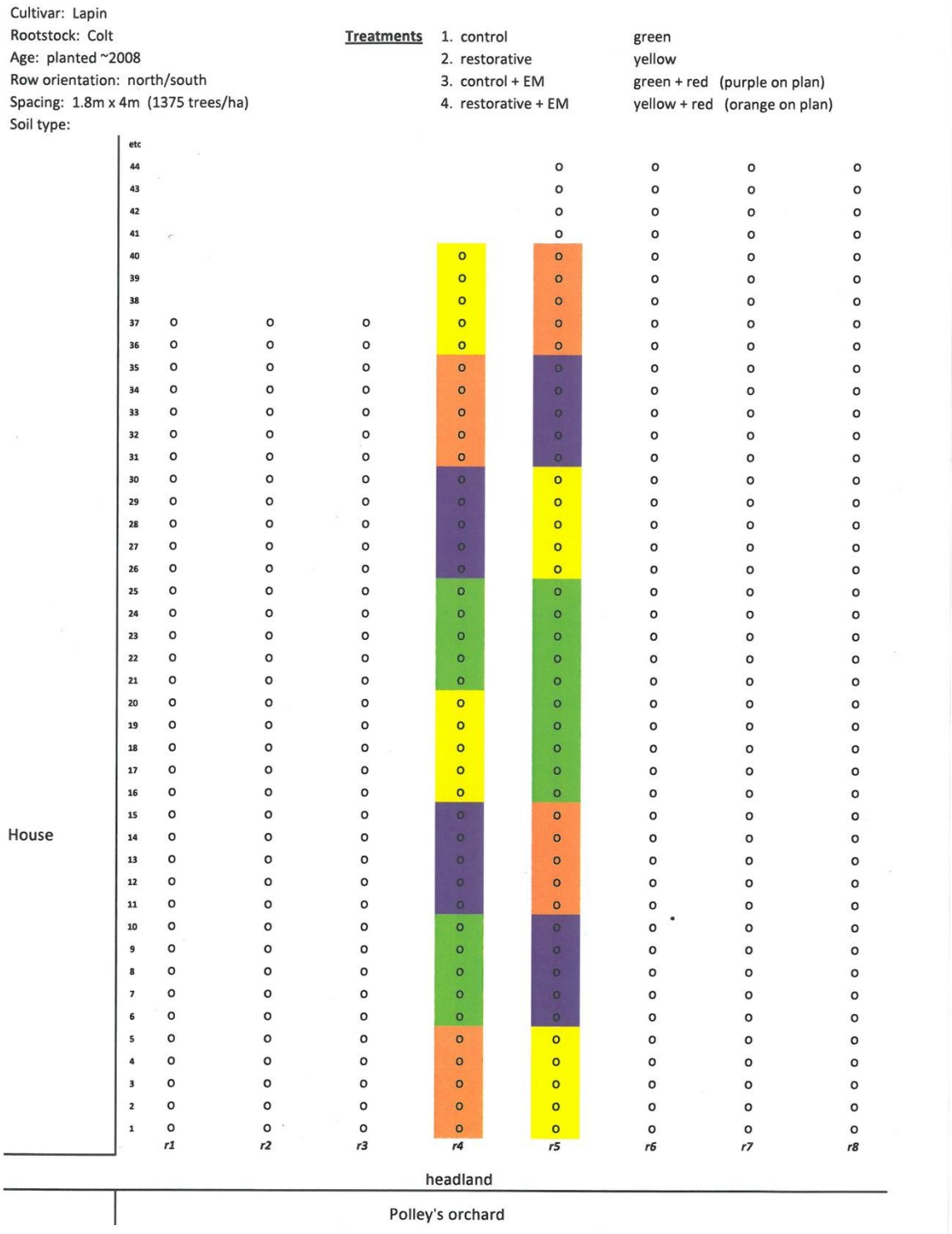

Figure 9.2 Rosegarland (Hansen Orchards) map

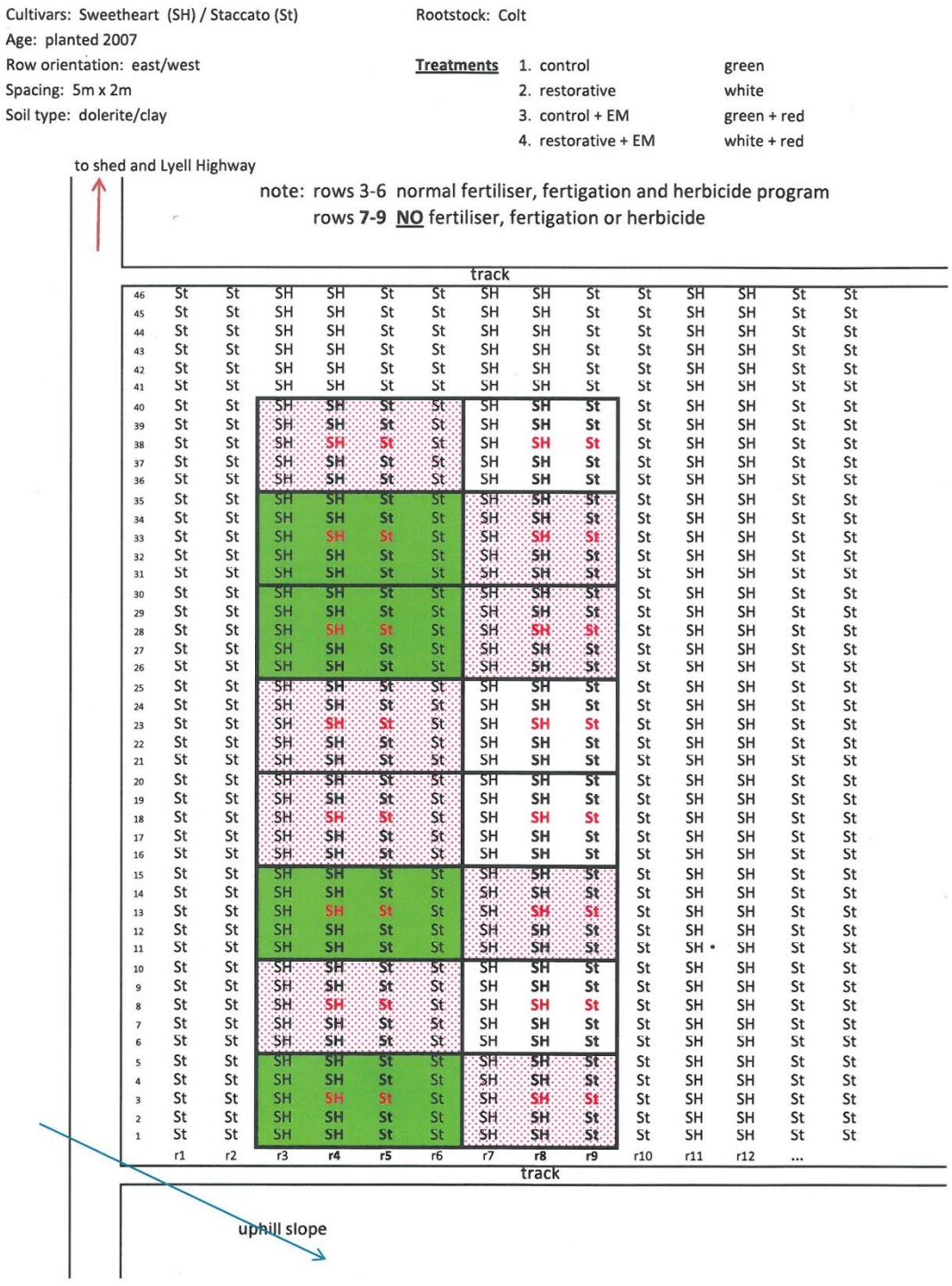

Figure 9.3 Lucaston Orchard (apples)